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VIROSES OF THE BEAN

WALTER H. PIERCE[1]

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In the course of studies undertaken on the relative resistance of bean varieties to mosaic, it became apparent that the susceptibility of the bean to more than one virus needed further study. The results presented in this paper show that, as in potato, tobacco, and many other plants, several distinct viruses infect bean. This fact has an important bearing on future investigations of any phase of the bean mosaic problem. The present paper reports results of a comparative study of five viruses which infect this plant.

EARLIER INVESTIGATIONS

The early studies on bean mosaic, begun by Reddick and Stewart (30, 31, 32, 33) in 1917, were concerned with a virus transmissible to many varieties of *Phaseolus vulgaris* L., and one which was carried in the seed. Furthermore it was shown by the same authors that the variety Robust was highly resistant to the disease. In subsequent studies on bean mosaic it seems apparent that Pierce and Hungerford (27), Fajardo (10, 11), and Nelson (26) were concerned with the same virus.

In the meantime a considerable number of other leguminous plants were shown by other investigators (5, 7, 8, 9, 15) to be susceptible to mosaic diseases, but strangely enough none was shown to be identical with the mosaic on bean nor were any of the viruses concerned transmitted to the bean. It was not until Carsner (6) showed that the virus of curly top of sugar beet would produce symptoms on bean that any positive evidence of the susceptibility of the bean to more than one virus was demonstrated. Later, Wingard (35) showed that the tobacco ring-spot virus would infect bean as well as a few other leguminous hosts. Price (28) showed that the ordinary tobacco-mosaic virus would produce local lesions on bean. Nelson (26), on the basis of symptom expression, claimed the existence of another virosis on the bean which he referred to as "rugose mosaic." Zaumeyer

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(36) has recently reported the transmission of certain legume-mosaic viruses to the bean, but their identity was not established.

For a more extensive review of the literature on the common type of bean mosaic reference may be made to Nelson's (26) recent publication.

EXPERIMENTAL MATERIALS AND METHODS

Source of Viruses Studied

Common Bean-Mosaic Virus. This is the virus of the disease originally described by Stewart and Reddick (33). It is by far the most common and prevalent virus of the bean. For these studies it was secured from mosaic seedlings grown from infected seed. Its correct identity is based on descriptions of the disease by Stewart and Reddick (33) and others, on the properties of the virus as described by Fajardo (11), and on its transmission by seed (32). This virus is designated as *bean virus 1* and is described in detail later in the paper.

Yellow Bean-Mosaic Virus. This virus was secured from a plant of the Red Valentine variety, grown in the field at Madison, Wisconsin, in 1931. Its specificity was first suspected not only from the fact that it produced symptoms distinct from that of the common bean-mosaic virus, but also because it affected the varieties, Corbett Refugee, Great Northern UI No. 1, and Robust, which are resistant to common mosaic. This virus is designated as *bean virus 2* and also is described in detail later.

Alfalfa-Mosaic Virus. This virus was secured from a strain of Turkestan alfalfa grown by Dr. F. R. Jones. The affected plants had been removed from the field and potted in the greenhouse in the autumn of 1931. The plants were decidedly dwarfed and the leaves distinctly mottled. The extracted juice from such plants, when inoculated to bean, produced necrotic lesions on certain varieties.

This disease on alfalfa appeared to be distinct from the mosaic on the same species described by Weimer (34). With his permission the virus he described in designated as *alfalfa virus 1,* while that from alfalfa used by the writer is herein referred to as *alfalfa virus 2.*

Ordinary Tobacco-Mosaic Virus. This virus, described by Johnson (21) as *tobacco virus 1,* has been shown by Price (28) to produce local necrotic lesions on bean. Inoculum for these studies was secured by the writer from Dr. James Johnson.

Tobacco Ring-Spot Virus. Ring-spot of tobacco was first described by Fromme and Wingard (13). The properties and host range of the virus were discussed in later papers from the same laboratory (14, 29, 35). Wingard (35) reported its successful transmission to bean. It was secured by the writer from Johnson and Grant (22), who in turn had received it from Wingard.

Source of Bean Varieties

Twenty-four varieties of common bean were used, all of which are listed in Table 1. Many of these were furnished by Associated Seed Growers Inc., New Haven, Connecticut. Corbett Refugee is a selection, resistant to common bean mosaic, derived from a single plant of Refugee Green[2] variety by Ralph Corbett of the Sioux City Seed Company, Sioux City, Iowa, who supplied seed to the writer. Great Northern UI No. 1 was selected for mosaic resistance from commercial Great Northern by the writer at the Idaho Agricultural Experiment Station, where seed of both these strains was secured for this work. Robust and Mexican Tree varieties were supplied by the Michigan Agricultural Experiment Station. Seed of Refugee Green variety was furnished by E. J. Renard of the Canners Seed Corporation, Beaver Dam, Wisconsin. Wisconsin Hybrid Wax No. 536 was secured from G. H. Rieman, formerly of the United States Department of Agriculture. Seeds of other leguminous plants were secured from various sources and, as far as could be determined, were true to name.

Methods

The studies were carried out partly in the greenhouse, where the temperature was usually held at 24–28° C., and in field plots on the experiment station farm at Madison, Wisconsin. Inoculum was prepared by crushing leaves and stems of infected plants in a sterile mortar and straining through cheesecloth. Bean plants were inoculated in the young two-leaf stage by rubbing the upper surfaces of the first leaves with a small piece of cheesecloth that had been immersed in the inoculum. Other host plants were inoculated by the same method. Where symptoms on inoculated plants were very mild or completely masked, the presence or absence of the virus was determined in some instances by extracting the juice and inoculating to healthy Refugee Green plants grown from a mosaic-free stock of seed, and used for this purpose because of its high susceptibility to all the viruses studied.

The properties of the viruses were determined on greenhouse-grown plants by the same methods described by Johnson and Grant (22).

In the experiments on aphid transmission the method described by Hoggan (18) was used.

EXPERIMENTAL RESULTS

The comparative studies of the five viruses under consideration were first based upon symptom expression and varietal susceptibility on 24 varieties of the common bean. Further points of differentiation are to be found

[2] This variety is known in the seed trade as Refugee Green, Stringless Green Refugee, and Late Stringless Refugee Green Pod.

in the results presented on the properties of the viruses *in vitro* and on their modes of transmission. The susceptibility or resistance of a number of other leguminous species also was determined. It will be seen that host reaction becomes an important criterion of distinction between the viruses.

Symptom Expression

Common Bean Mosaic (*bean virus 1*).—On account of the known variation in susceptibility of bean varieties to *bean virus 1,* the study of symptom expression on the 24 varieties was simplified by dividing them into 3 classes according to their resistance to *bean virus 1.* Class I consists of susceptible varieties on which pronounced symptoms are produced. Class II may be defined as the tolerant class. These varieties are infected as readily by inoculation as those in Class I but do not ordinarily develop marked symptoms. However, the virus may be recovered readily from inoculated plants even when the disease is completely masked. Class III comprises those varieties that are immune from *bean virus 1.* They develop no symptoms on inoculation and the virus cannot be recovered from inoculated plants. In table 1 the 24 varieties are listed in their respective classes. The symptoms of common bean mosaic (*bean virus 1*) have been described by Stewart and Reddick (33), Barss (4), Pierce and Hungerford (27), Fajardo (10), and Nelson (26); but, since both *bean virus 1* and *bean virus 2* cause systemic infection with some overlapping of symptoms, the signs of both will be described on Refugee Green and Mexican Tree from Class I, Giant Stringless and Brittle Wax from Class II, and Corbett Refugee, Robust, and Great Northern UI No. 1 from Class III.

When the primary leaves of the susceptible Refugee Green are inoculated with *bean virus 1,* symptoms first appear as a slight chlorosis of the first trifoliate leaf, which tends to curl but, ordinarily, shows no mottling. The leaflets of the second trifoliate leaf become somewhat elongated, with the lamina curled downward. Mottling occurs in various patterns, but, commonly, the background is of a somewhat lighter green than normal, with dark green areas interspersed on the light green field. The dark green often appears as a band along the midvein (Fig. 1, D). Under greenhouse conditions, during the winter months, the mottling may be almost entirely masked and only a slight curling or a faint chlorosis gives evidence of the presence of the virus. Under field conditions mottling may be accentuated to the extent that the dark green areas become savoyed (Fig. 1, E). The downward curling is a rather constant feature and is one of the significant ways in which it differs from yellow bean mosaic. The sequence of symptoms, as growth continues, may show considerable variation, but the stunting, curling, and mottling so evident on diseased plants during their first 2 months of growth, usually become less pronounced as the plants approach

TABLE 1.—*Comparison of the susceptibility of certain bean varieties to common bean mosaic (bean virus 1)*

Class	Variety	Inoculated in the greenhouse		Inoculated in the field, 1931		Natural infection in the field, 1932	
		Inoculated	Infected	Inoculated	Infected	Plants	Infected
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
I. Susceptible	Dwarf Horticultural	35	90	37	97	124	95
	Comm. Great Northern	51	82	32	79	123	86
	Mexican Tree	53	85	37	94	176	86
	Red Kidney	40	92	20	85	118	99
	Red Valentine	46	91	43	100	104	95
	Refugee Green	180	90	57	96	784	93
	Refugee Wax	40	92	53	94	123	100
	Well's Red Kidney	31	84	....	....	28	75
II. Tolerant	Black Valentine	40	77	40	95	105	89
	Bountiful	40	85	33	93	122	87
	Brittle Wax	46	82	57	84	125	75
	Burpee's S. G. Pod	42	83	36	94	129	79
	Davis Wax	35	91	36	83	101	76
	Full Measure	36	80	34	93	119	83
	Giant S. G. Pod	49	80	68	91	115	89
	Imp. Kidney Wax	40	75	64	78	148	73
	Longfellow	40	70	46	79	114	82
	Pencil Pod	40	77	44	86	156	83
	Sure Crop Wax	40	85	42	89	121	96
	Unrivalled Wax	52	8	....	....	68	4
III. Immune	Corbett Refugee	192	0[a]	56	0	1110	1+[b]
	Great Northern UI No. 1	90	0[a]	100	2[b]	117	3–[b]
	Robust	119	0[a]	154	4[b]	87	2+[b]
	Wis. Hybrid Wax No. 536	70	2–	112	9[b]	57	3+[b]

[a] Transfer inoculations to Refugee Green demonstrated that *bean virus 1* was not present.

[b] Test inoculations in the greenhouse showed the mosaic infection on these varieties to be due to *bean virus 2* and not to *bean virus 1*.

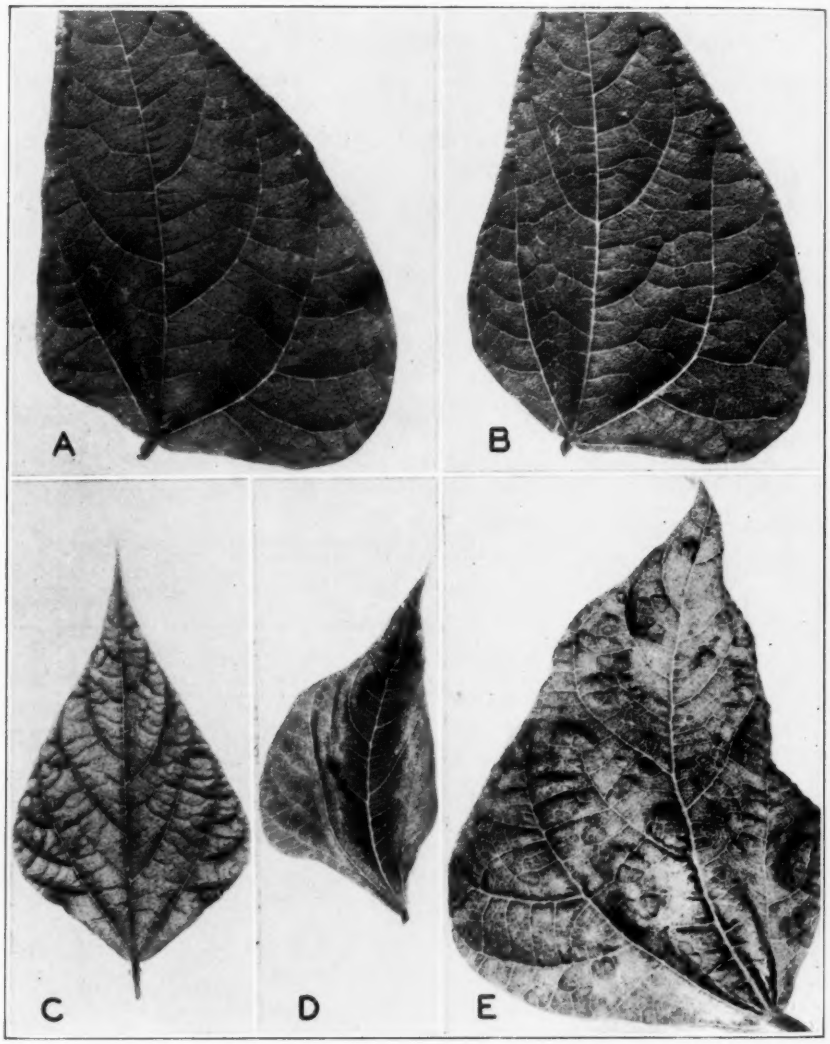

FIG. 1. Variation in leaf symptoms exhibited by different varieties of bean inoculated with *bean virus 1*. A. Corbett Refugee noninoculated control. B. Corbett Refugee inoculated but showing no symptoms. C. Giant Stringless, showing no symptom other than reduction in size. D. Refugee Green showing chlorosis with dark green area along midvein. E. Refugee Green with severe symptoms.

maturity. While yield is reduced and maturity delayed, the effects are not nearly so pronounced with *bean virus 1* as with *bean virus 2*.

On the Mexican Tree variety the symptoms are much like those described for Refugee Green, except that in the second trifoliate leaf the dark green

areas more commonly follow along the midribs and extend along the lateral veins towards the margin of the leaflets, whereas the intervenal areas are light green in color.

On Giant Stringless and Brittle Wax varieties the symptoms are very mild under greenhouse conditions (Fig. 1, C). In some cases dark green areas can be detected along the veins of the second trifoliates of plants inoculated in the primary leaf stage. In the subsequently developing leaves it is often impossible to detect mosaic symptoms. To determine infection on these varieties it is often necessary to make transfer inoculations to a more susceptible variety, such as Refugee Green.

Corbett Refugee, Robust, and Great Northern UI No. 1 are apparently immune from *bean virus 1*. Repeated inoculations failed to produce symptoms (Fig. 1, A, B). Juice extracted from inoculated plants of the last-named varieties fails to produce symptoms on the Refugee Green variety.

Yellow Bean Mosaic (*bean virus 2*).—On Refugee Green, *bean virus 2* is much more virulent than the former virus (Fig. 2, A–C). Inoculation

FIG. 2. Refugee Green plants six weeks after inoculation. A. Control inoculated with water. B. Inoculated with *bean virus 1*. The leaves are mottled but there is little reduction in size of plants. C. Inoculated with *bean virus 2*. Note extreme reduction in size of the plant.

on the primary leaves usually produces a distinctive drooping of the developing trifoliate leaf. Each leaflet is not only curled downward but, in addition, is definitely pointed downward from the point of attachment to the petiole. This is a very simple method of distinguishing between *bean virus 1* and *bean virus 2*. The surfaces of the leaflets are slightly irregular, and small, light yellow spots soon develop in the dark green background of the first trifoliate. The yellowing gradually spreads over the entire surface, causing the leaflets to become more or less chlorotic. In these early stages the young growth has a tendency to become brittle. The first trifoliate leaflets do not remain curled downward, but, as they enlarge, become slightly concave on their upper surfaces and take on a glossy appearance.

On the third and fourth trifoliate leaves there is a very distinct mottling of yellow green and dark green areas, which stand out in greater contrast to each other than is the case with common bean mosaic (Fig. 3, F). The

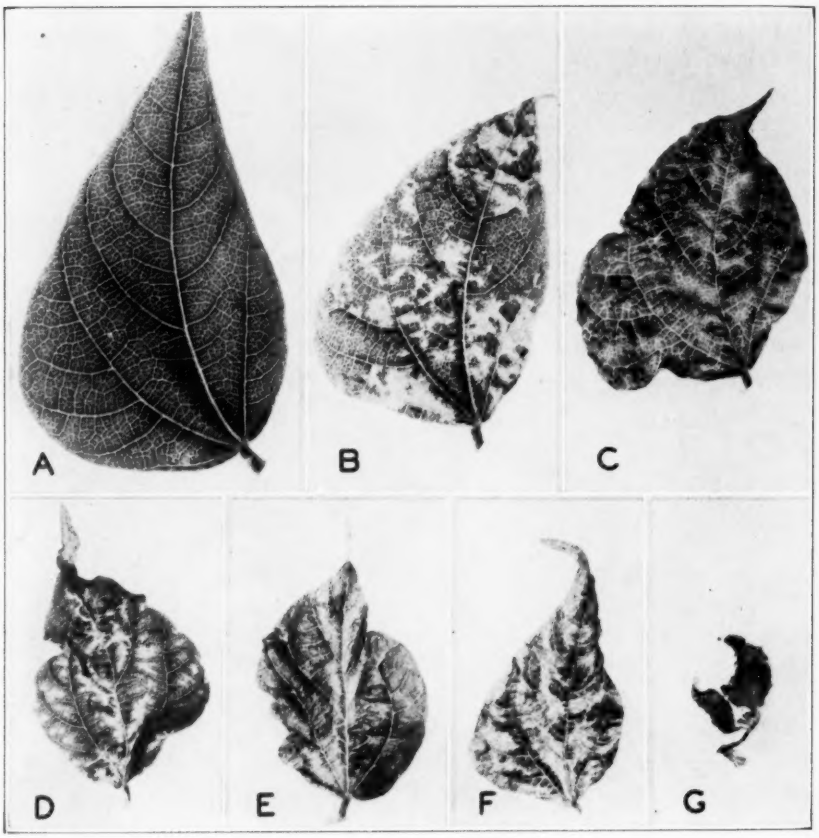

FIG. 3. Variation in leaf symptoms exhibited by different varieties of bean infected with *bean virus 2*. A. Corbett Refugee healthy. B. Corbett Refugee showing mottling. C. Robust showing mottling. D. Brittle Wax with mottling and malformation. E. Giant Stringless with mottling and malformation. F. Refugee Green showing mottling and reduced size. G. Mexican Tree with complete killing of leaflets.

downward curling usually associated with the latter is not a constant characteristic of yellow bean mosaic in the later stages of development. In plants affected with yellow bean mosaic, symptoms do not become masked in their later stages of growth, but the mottling becomes more pronounced as the season progresses. Plants become decidedly stunted and bushy due to a reduction in the length of the internodes and a proliferation of branches. Maturity is greatly delayed and production of pods is greatly reduced.

When the Mexican Tree variety is inoculated on the primary leaves a lethal effect upon the developing trifoliate leaves is produced (Fig. 3, G). Usually the young epicotyl is entirely killed. In some cases growth continues from the buds in the axils of the primary leaves, but the plants never develop sufficiently to blossom and set fruit. The secondary growth is decidedly proliferated, mottled, and malformed.

The leaves of Giant Stringless and Brittle Wax have a tendency to curl and become otherwise malformed. The lighter-colored portions of the mottling are in some cases devoid of all green color (Fig. 3, D, E). These light-colored areas are usually depressed somewhat from the convex surface of the curled leaf. Stunting and proliferation are not so marked as on Mexican Tree. The pods of affected plants become distorted and mottled.

The first symptoms following inoculation on Corbett Refugee, Robust, and Great Northern UI No. 1 are similar to those described on Refugee Green. In the later stages of development, however, the plants are not so severely dwarfed and, even though mottling is often pronounced, the set of pods is not appreciably reduced (Fig. 3, A–C). There is no curling of the leaves.

Alfalfa Virus 2. This virus produces local necrotic lesions on Refugee Green beans within 2 days following inoculation. The lesions appear first as minute light brown spots about $\frac{1}{2}$ mm. wide. They gradually widen and attain a maximum diameter of 1 to 2 mm. in 6 to 8 days. The color of fully formed lesions is chocolate brown. The lesions formed on Red Valentine and certain other varieties are slightly larger than those on Refugee Green (Fig. 4, A). The disease does not become systemic on any of the varieties of *Phaseolus vulgaris* tested.

Tobacco Virus 1. This virus also produces local necrotic lesions on Refugee Green beans. Price (28) described the lesions caused by *tobacco virus 1* as from less than $\frac{1}{2}$ to 1 mm. wide and consisting of pale necrotic areas surrounded by a ring of dark red tissue. The writer obtained similar results. The lesions develop more slowly and do not become so large as those caused by *alfalfa virus 2* (Fig. 5, C). No systemic effect on the plants was observed.

Tobacco Ring-spot Virus. The symptoms of tobacco ring spot on bean also vary somewhat with different varieties. The symptoms on Robust and Mexican Tree consist of local lesions that are necrotic throughout (Fig. 5, B). These lesions gradually extend until they coalesce and the leaf collapses. On other varieties they are much less distinct, appearing as necrotic rings in the normal tissue or as a vein necrosis (Fig. 4, B). Following the development of local lesions on the inoculated leaves, symptoms appear in a few days on the young noninoculated leaves. This systemic infection causes the death of the entire plant. Corbett Refugee, Robust,

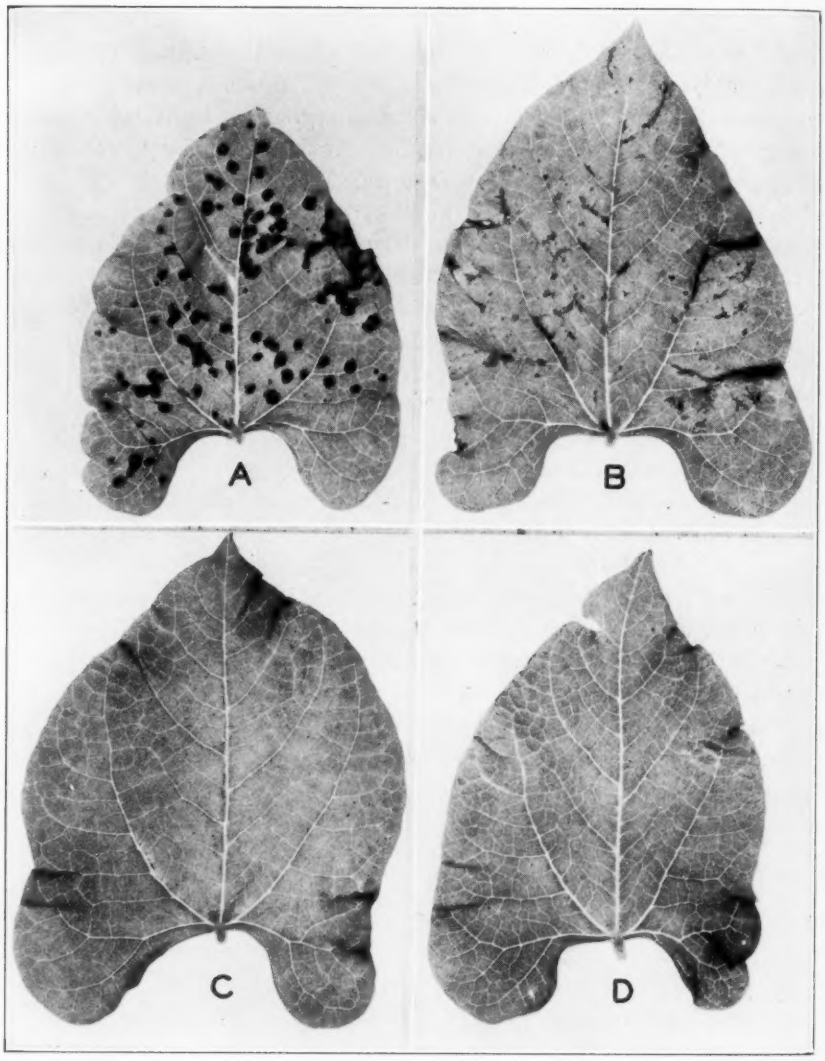

FIG. 4. Primary leaves of Red Valentine bean showing various symptoms. A. Necrotic local lesions caused by inoculation with *alfalfa virus 2*. B. Local lesions and vein necrosis caused by tobacco ring-spot virus. C. No local lesions from inoculation with *tobacco virus 1*. D. Noninoculated control.

and Mexican Tree, however, seldom become systemically infected with ring spot.

Resistance and Susceptibility in Bean Varieties

Common Bean Mosaic (bean virus 1). All 24 varieties were inoculated in the greenhouse and further tests were made in the field. In the green-

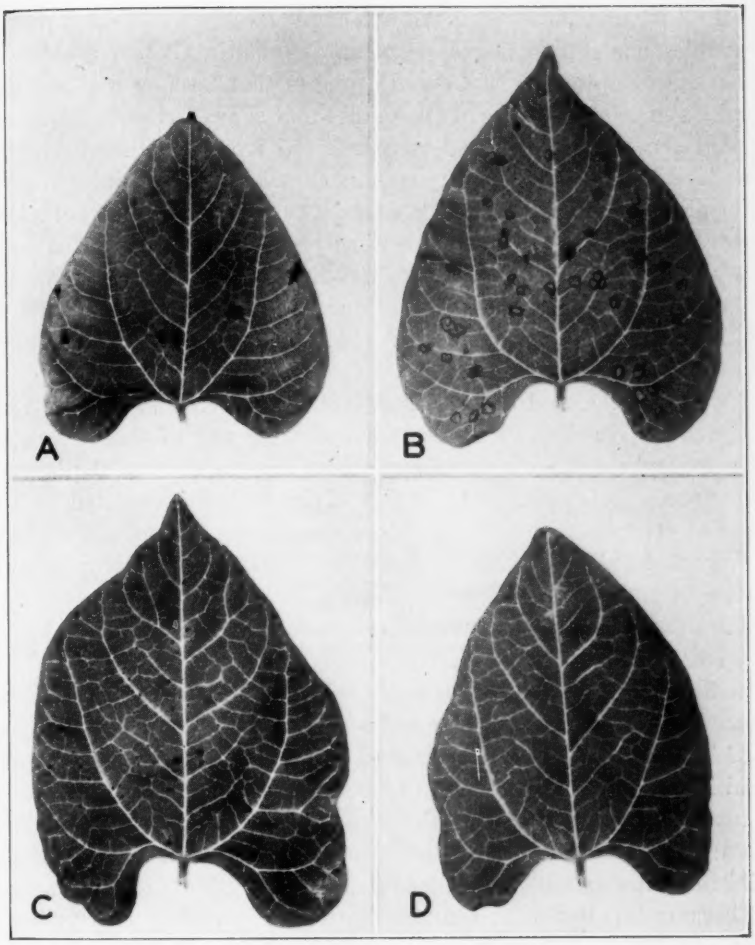

FIG. 5. Primary leaves of Mexican Tree bean showing necrotic local lesions produced by inoculations with different viruses. A. *Alfalfa virus 2*. Only a few lesions. B. Tobacco ring-spot virus. C. *Tobacco virus 1*. D. Noninoculated control.

house each plant was inoculated twice, once on the primary leaves, and once on the first trifoliate leaf. In the summary of results given in table 1, the varieties are divided into the 3 classes discussed under symptoms. These will be referred to for convenience as Class I, susceptible; Class II, tolerant; and Class III, immune. They are probably similar to those classes designated by Reddick and Stewart (30, 31) who divided a much longer list of varieties into "highly susceptible," "intermediate," "resistant," and "immune" classes.

The percentage of plants that showed symptoms in Classes I and II is generally high, with the exception of Unrivalled Wax. The distinction be-

tween these groups, however, is not one of percentage of infected plants but that of tolerance and disease expression. Plants in Class I usually exhibited marked symptoms. In Class II, plants that became diseased showed only mild symptoms. Some of the plants that showed slight symptoms and some that showed no symptoms were selected from inoculated populations of Brittle Wax and Giant Stringless varieties. When the juice from these was inoculated into healthy Refugee Green plants, a high percentage of the plants were shown to have had the virus present, regardless of whether or not they showed visible symptoms of mosaic.

In Class III, Robust, Corbett Refugee, and Great Northern UI No. 1 plants showed no mosaic symptoms whatever (Fig. 1, A, B). Juice was extracted from each of 68 inoculated Corbett Refugee plants, 25 of Great Northern UI No. 1, and 30 of Robust and transferred to healthy Refugee Green plants. No signs of mosaic resulted from any of these inoculations. Wisconsin Hybrid Wax showed symptoms on one of the 70 plants used. Juice extracted from 17 plants in this variety when inoculated to healthy Refugee Green plants failed to produce the disease in any case except that of juice extracted from the one plant that had shown symptoms. This exception is not clear but since it occurred in only one plant, which might have been the result of seed admixture or even a field cross, the variety is placed tentatively in Class III.

The field inoculations in 1931 and exposure to natural infection in 1932 confirmed the results from the greenhouse. Varieties in Class III all remained free from common bean mosaic, although a small percentage showed natural infection with yellow bean mosaic. Each of these plants was tested by transferring juice to healthy Refugee Green and was shown to be affected with only yellow bean mosaic. Of Class II all showed a high percentage of mild infection except Unrivalled Wax. Three plants of this variety out of 68 became infected with common bean mosaic and 2 with yellow bean mosaic. In Class I all varieties were severely diseased.

Yellow Bean Mosaic (*bean virus 2*). Greenhouse inoculations showed that all varieties were susceptible to yellow bean mosaic (Table 2). However, the data lend support to the classification scheme set up with respect to varietal susceptibility to *bean virus 1*. The immune varieties of Class III, though somewhat dwarfed at first, and later characterized by a distinct mottling of the leaves, usually developed into full-size plants. Serious injury in the field was not observed and the natural occurrence of *bean virus 2* on varieties of Class III was limited to a small percentage of plants. In a Corbett Refugee planting of 1110 plants in 1932 only 13 were affected with yellow bean mosaic. Only small percentages of infected plants occurred in the Great Northern UI No. 1 and Robust varieties grown at Madison, Wisconsin, in 1931 and 1932. Yellow bean mosaic in the field is par-

TABLE 2.—*Relative susceptibility of varieties of bean to bean virus 2, alfalfa virus 2, tobacco virus 1, and tobacco ring-spot virus based on greenhouse inoculations*

Variety	Bean virus 2[a]		Alfalfa virus 2[b]			Tobacco virus 1[b]			Ring-spot virus		
	Total plants inoculated	Per cent systemically infected	Total plants inoculated	Per cent locally infected	Ave. no. lesions per leaf	Total plants inoculated	Per cent locally infected	Ave. no. lesions per leaf	Total plants inoculated	Per cent systemically infected	Per cent locally infected	Ave. no. lesions per leaf
Dwarf Horticultural	23	60	26	100	16	31	0	0	16	100	50	−2
Comm. Great Northern	41	27	26	50	−1	24	100	35	19	52	30	1
Mexican Tree	38	90	28	100	2	29	100	58	17	17	66	−2
Red Kidney	23	56	19	100	35	23	0	0	5	100	100	5
Red Valentine	27	96	28	100	50	28	0	0	17	100	100	20
Refugee Green	52	75	28	100	34	27	100	30	17	100	100	3
Refugee Wax	38	52	26	100	26	28	100	30	16	100	100	18
Well's Red Kidney	30	50	21	100	44	27	0	0	16	100	68	4
Black Valentine	39	66	26	100	32	32	0	0	19	100	100	13
Bountiful	20	60	24	100	23	30	0	0	16	100	100	30
Brittle Wax	42	78	21	100	50	21	0	0	13	100	100	20
Burpee's S. G. Pod	23	56	24	100	40	29	0	0	16	100	100	20
Davis Wax	31	42	12	100	32	16	0	0	6	100	100	13
Full Measure	20	95	26	100	40	27	0	0	18	100	100	15
Giant S. G. Pod	47	47	27	100	33	28	0	0	15	100	100	14
Imp. Kidney Wax	24	62	19	100	44	18	0	0	11	100	100	16
Longfellow	26	59	26	100	15	23	0	0	14	100	100	5
Pencil Pod	31	45	24	100	36	27	0	0	16	100	100	22
Sure Crop Wax	28	57	28	100	48	29	0	0	11	100	100	15
Unrivalled Wax	47	12	27	100	45	30	0	0	18	100	100	22
Corbett Refugee	48	50	26	20	−1	28	100	20	18	0	100	3
Great Northern UI No. 1	39	33	40	50	−1	21	90	23	16	94	50	−1
Robust	42	60	34	50	−1	31	100	35	17	12	100	6
Wis. Hybrid Wax No. 536	44	14	28	100	46	26	19	−1	15	100	50	−1

[a] No local necrosis produced by *bean virus 1* nor by *bean virus 2*.
[b] No systemic infection produced by *alfalfa virus 2* nor by *tobacco virus 1*.

ticularly noticeable on these varieties, since there is no confusion with common bean mosaic. It is more difficult to distinguish accurately between the two diseases on a variety like Refugee Green, which is susceptible to both. Most varieties in Class I were very severely stunted. In some cases they were killed outright as described earlier under symptoms on Mexican Tree variety. Red Kidney and Well's Red Kidney were exceptional in that they were more tolerant to yellow bean mosaic than most varieties in Classes II and III. In general, the varieties of Class II were less severely affected than were those of Class I.

Alfalfa Virus 2.—The data in table 2 indicate the percentage of plants infected and the average number of local necrotic lesions per leaf. All varieties showed some lesions. Of those highly resistant to *bean virus 1* and *bean virus 2*, Corbett Refugee, Great Northern UI No. 1, and Robust showed very few lesions on 50 per cent or less of the plants. Wisconsin Hybrid Wax showed no marked resistance. Of those varieties very susceptible to *bean viruses 1* and *2*, Great Northern and Mexican Tree were highly resistant to *alfalfa virus 2* (Fig. 5, A). Thus resistance to this virus is not correlated with that to the two bean-mosaic viruses.

Alfalfa Virus 1.—Price (28) tested a large number of bean varieties and found many that did not develop lesions when inoculated with this virus. Many of the varieties considered here (Table 2) showed complete resistance (Fig. 4, C). There is no correlation, however, between resistance to this virus with that to the two bean viruses. Many of the varieties susceptible to *tobacco virus 1* showed some resistance to *alfalfa virus 2* (Fig. 5, A, C), but here, again, the correlation is not complete.

Tobacco Ring-spot Virus.—As shown in table 2, all varieties are affected, usually with local necrotic lesions on the inoculated leaf and systemic necrotic symptoms on the younger portions of the plant. Corbett Refugee was the only one that showed no systemic infection, but a few local lesions were produced. Of the varieties Robust, Mexican Tree, and Great Northern only 12 to 52 per cent of the plants were systemically infected, and thus appeared to have some tolerance. All other varieties were severely diseased.

PROPERTIES

The properties of common bean mosaic were studied by Fajardo (11). His work has been repeated in part for the purpose of obtaining a direct comparison of the properties of *bean virus 1* with those of *bean virus 2*, *alfalfa virus 2*, and the tobacco ring-spot virus. The properties of *tobacco virus 1* were not studied, for they have received attention by Allard (1, 2, 3) and many others. The property tests on all the viruses were made in the same manner and under as uniform conditions as possible. In these tests five bean plants were used as a test unit. In the summary in tables 3

TABLE 3.—*Comparison of certain properties of bean virus 1, bean virus 2, and tobacco ring-spot virus, as determined by systemic infection on Refugee Green bean*

1934]

Resistance to chemicals[a]

TABLE 3.—*Comparison of certain properties of bean virus 1, bean virus 2, and tobacco ring-spot virus, as determined by systemic infection on Refugee Green bean*

Thermal death point				Longevity *in vitro*				Tolerance to dilution				Resistance to chemicals[a]				
Temp. °C.	Bean virus 1	Bean virus 2	Tobacco ring-spot virus	Time aged	Bean virus 1	Bean virus 2	Tobacco ring-spot virus	Dilution	Bean virus 1	Bean virus 2	Tobacco ring-spot virus	Chemical	Strength of chemical	Bean virus 1	Bean virus 2	Tobacco ring-spot virus
Inoc.	25[b]/19	25/15	10/10	None	25/20	25/21	10/10	None	25/17	25/17	10/10	None		25/21	25/16	10/10
Control	25/16	25/8		20 hours	25/15	25/9		1–10	25/20	25/17	10/10	Alcohol	25%	25/10	25/9	10/10
54	25/10	25/4		24 do	25/7	25/5	10/10	1–100	25/10	25/15	10/10	do	50%	25/0	25/0	10/10
56	25/0	25/0		28 do	25/2	25/3		1–200	25/8	25/11	----	do	75%	----	----	10/5
58			10/10	32 do	25/0	25/0		1–400	25/5	25/9	----	Nitric acid	1–500	25/16	25/13	10/10
62			10/7	3 days			10/10	1–800	25/3	25/3	----	do	1–200	25/0	25/0	10/6
64			10/0	5 do			10/5	1–1000	25/2	25/0	10/3	do	1–100	----	----	10/0
66				7 do			10/4	1–2000	25/0	25/0	10/0	Formaldehyde[c]	1–2000	25/13	25/8	----
				8 do			10/1					do	1–1000	25/7	25/0	5/5
				9 do			10/0					do	1–500	25/0	25/0	10/10
												do	1–200			10/1
												do	1–100			10/0

[a] 30-minute treatments.
[b] Upper figure is number of plants inoculated; lower figure, number of plants infected.
[c] Concentrated solution, which contains approximately 37 per cent formaldehyde.

and 4 the results of 5 tests for each property determined have been totalled. It should be pointed out that minor variations occurred in the individual trials that are not always apparent in the summarized results. The properties of *alfalfa virus 2* were tested by its ability to produce local lesions on Refugee Green bean (Table 4). One-half of the tobacco ring-spot virus determinations were based upon local infection on Henderson Bush Lima (Table 4), and one-half on systemic infection on Refugee Green bean (Table 3). In some of the trials with ring spot, Refugee Green beans appeared to be slightly more sensitive to systemic infection than was Henderson Bush Lima bean to local infection.

Thermal Inactivation Point. (Tables 3 and 4). The thermal inactivation point of *bean virus 1* was found to approximate the upper limits set by Fajardo (11). *Bean viruses 1* and *2* were both inactivated at 56° to 58° C. when exposed at this temperature for 10 minutes. (Table 3). *Alfalfa virus 2* was inactivated at 62° to 64° C. (Table 4). Inactivation of the tobacco ring-spot virus at 66° C. is in general agreement with the determinations by Henderson and Wingard (17) and Johnson and Grant (22).

The thermal death point of *tobacco virus 1,* being generally accepted at about 90° C., it is apparent that, with the exception of the close similarity between the 2 bean viruses, the 5 viruses considered in this paper have rather distinct thermal properties.

Resistance to Aging in Vitro. (Tables 3 and 4). *Bean viruses 1* and *2,* in all trials, lost their infectivity after aging *in vitro* 24 to 32 hours. Fajardo (11) had previously shown *bean virus 1* to be inactivated at 24 hours. *Alfalfa virus 2* and the tobacco ring-spot virus were both inactivated after 7 to 9 days. Priode (29) found the ring-spot virus to be still infective after aging 7 days at 10° C. Johnson and Grant (22) found it to be inactivated in 5 to 6 days at room temperature. All the above viruses afford a striking contrast to *tobacco virus 1,* which is known to remain infective *in vitro* for two years or more (2, 21).

Tolerance to Dilution. (Tables 3 and 4). Dilution tests showed no significant differences between *bean virus 1, bean virus 2, alfalfa virus 2,* and the tobacco ring-spot virus. All lost their infectivity at dilutions greater than 1 to 1000. Infection with *bean virus 2* was not obtained with dilutions greater than 1 to 800, but this cannot be considered a significant difference from the 1 to 1000 dilution noted in this study and also by Fajardo (11) for *bean virus 1.* No infection was obtained with the tobacco ring-spot virus at dilutions greater than 1 to 1000. Henderson and Wingard (17), however, obtained a trace of infection at 1–10,000. All the above viruses are distinct from *tobacco virus 1,* which has been shown by Allard (1) and by Johnson and Grant (22) to be infective at a dilution of 1 to 1,000,000.

TABLE 4.—*Comparison of certain properties of alfalfa virus 2 and tobacco ring-spot virus, as determined by the production of local lesions on bean*

Thermal death point			Longevity *in vitro*			Tolerance to dilution			Resistance to chemicals[a]			
Temp. ° C.	Alfalfa virus 2	Tobacco ring-spot virus	Time aged days	Alfalfa virus 2	Tobacco ring-spot virus	Dilution	Alfalfa virus 2	Tobacco ring-spot virus	Chemical	Strength of chemical	Alfalfa virus 2	Tobacco ring-spot virus
Inoc. control	2102[b]	677[c]	None	4185[b]	198[c]	None	3904[b]	300[c]	None		4919[b]	277[c]
58	1701	.....	1	3051	109	1–10	1952	38	Alcohol	25%	980	103
60	1084	.....	3	855	31	1–100	368	14	do	50%	50	41
62	379	34	5	408	6	1–200	180	.....	do	75%	0	0
64	0	5	7	62	0	1–400	89	.....	Nitric	1–500	25	145
66	.....	0	8	5	0	1–800	31	.....	acid	1–200	0	19
			9	0	0	1–1000	13	0	do	1–100	.....	0
						1–2000	1	0	Formalde-	1–1000	.....	161
									hyde	1–500	1675	36
									do	1–200	83	0
									do	1–100	0	0

[a] 30-minute treatments.

[b] Figures are total lesions produced on 50 inoculated primary leaves of Refugee Green bean.

[c] Figures are total lesions produced on 20 inoculated primary leaves of Henderson Bush Lima.

Resistance to Chemicals. (Tables 3 and 4). Allard (2), Johnson (21), and Johnson and Grant (22) have shown that *tobacco virus 1* has a high resistance to various chemical treatments. Fajardo (11) obtained inactivation of *bean virus 1* with 25 per cent alcohol for 30 minutes. In the present investigation *bean virus 1* withstood treatment with 25 per cent alcohol, but was always inactivated by 50 per cent alcohol. *Bean virus 2* also was inactivated by 50 per cent alcohol and *alfalfa virus 2* was inactivated by 75 per cent alcohol. The tobacco ring-spot virus was not inactivated by 75 per cent alcohol when the inoculation tests were made on Refugee Green beans, but the tests on Henderson Bush Lima gave no local lesions after treatment with this strength of alcohol. The tobacco ring-spot virus withstood a 30-minute treatment with nitric acid, 1–200, but *bean viruses 1* and *2* and *alfalfa virus 2* were all inactivated at this concentration. In the trials of Johnson and Grant (22) *tobacco virus 1* was not inactivated by nitric acid, 1–200, for 48 hours.

The infectivity of the viruses in question was destroyed by 30-minute treatments with 37 per cent formaldehyde at concentrations as follows: *bean virus 1* at 1–500; *bean virus 2* at 1–1000; *alfalfa virus 2* and tobacco ring-spot virus at 1–100. The tobacco ring-spot virus, however, did not produce local lesions on Henderson Bush Lima after treatment with 1–200 formaldehyde.

Transmission

By Insects. Transmission of bean mosaic (*bean virus 1*) by aphids was accomplished by Nelson (25) with the potato aphid, *Macrosiphum solanifolii* Ashm. Fajardo (10) reported successful transmission with *Aphis rumicis* L. and *Myzus persicae* Sulz. Zaumeyer (37), in addition to the above, obtained transmission with *Illinoia pisi* Kalt., *Aphis gossypii, Brevicoryne brassicae, Hyalopterus atriplicis,* and *Macrosiphum ambrosia.*

The writer secured transmission of *bean viruses 1* and *2* with both the pea aphid *Illinoia pisi* and the potato aphid, *Macrosiphum solanifolii,* thus confirming the previous reports of aphid transmission of *bean virus 1.* Systemic infection of *alfalfa virus 2* on red clover, white clover, and Perfection peas, and local infection on Refugee Green beans was obtained with the pea aphid, *Illinoia pisi.*

No aphid transmission tests were made with the tobacco ring-spot virus nor with *tobacco virus 1.* Hoggan (18, 19) has shown that *tobacco virus 1* is not transmitted by the peach aphid *Myzus persicae* and that *Myzus pseudosolani, Macrosiphum solanifolii,* and *Myzus circumflexus* are able to transmit *tobacco virus 1* from tomato to tobacco but not from tobacco to tobacco. Fenne (12) obtained negative results in attempts to transmit tobacco ring-spot virus by means of tobacco flea beetle, cucumber flea beetle, leaf hopper, aphis, firefly, and tobacco hornworm.

Through Seed. That the virus of common bean mosaic (*bean virus 1*) is transmitted through the seed of the common bean was first proved by Reddick and Stewart (32). Subsequent investigations by Pierce and Hungerford (27), Fajardo (10), and Nelson (26) have shown that, when large populations of seed from diseased plants are tested, from 30 to 50 per cent diseased seedlings are produced. Similar percentages were obtained in the present study.

In a total of 6532 seedlings grown from seed of plants infected with *bean virus 2* none were found to be affected with yellow bean mosaic (Table 5). These seedlings were grown from seed of varieties susceptible to both *bean virus 2* and *bean virus 1* and of varieties susceptible only to *bean virus 2*. They included seed from naturally infected plants from different

TABLE 5.—*Results of tests on seed transmission of bean virus 2*

Variety	History of infected plants from which seed was obtained	Number of seedlings grown	Number infected with bean virus 2
Corbett Refugee	Naturally infected in field. Billings, Montana. 1931.	340	0
	Naturally infected in field. Madison, Wisconsin. 1932.	2469	0
	Artificially infected in field. Madison, Wisconsin. 1932.	64	0
	Artificially infected in greenhouse. Madison, Wisconsin. 1931–32.	140	0
Great Northern UI No. 1	Naturally infected in field. Twin Falls, Idaho. 1931.	576	0
	Naturally infected in field. Madison, Wisconsin. 1931.	1171	0
	Artificially infected in greenhouse. Madison, Wisconsin. 1931.	15	0
Robust	Naturally infected in field. Madison, Wisconsin. 1931.	1023	0
	Artificially infected in field. Madison, Wisconsin. 1932.	73	0
	Artificially infected in greenhouse. Madison, Wisconsin. 1931–32.	72	0
Wis. Hybrid Wax No. 536	Artificially infected in field. Madison, Wisconsin. 1932.	31	0
Varieties susceptible to bean virus 1	Artificially infected in field. Madison, Wisconsin. 1932. Naturally infected with bean virus 1.	506	0
Manchu soybean	Artificially infected in greenhouse. Madison, Wisconsin. 1932.	52	0
	Total	6532	0

sources, as well as seed from plants artificially infected in the greenhouse with *bean virus 2*.

It is of interest to note the outcome of tests made with seeds obtained from plants infected with both *bean virus 1* and *bean virus 2*. A total of 506 seedlings (Table 5) were grown from seed harvested from field-grown plants that had been artificially infected with *bean virus 2* and naturally infected with *bean virus 1*. No evidence of the transmission of *bean virus 2* was obtained. However, a small percentage of the 506 seedlings exhibited symptoms of common bean mosaic; and transfer inoculations established conclusively that these mosaic plants were caused by the virus of common bean mosaic alone. The nontransmissibility of *bean virus 2* through the seed is one of the chief points of difference between it and *bean virus 1*. The results of a test on transmission of *bean virus 2* through the seed of soybean is included in table 5. None of the seedlings was infected.

In one trial of 31 seedlings, grown from the seed obtained from Manchu soybean plants that had been artificially infected with *alfalfa virus 2*, none of the seedlings was infected. Gardner and Kendrick (15) described a mosaic of soybean, transmitted through the seed, but they were unable to infect varieties of *Phaseolus vulgaris* with their soybean-mosaic virus (23). It appears, therefore, that their soybean mosaic was not caused by *bean virus 2* nor by *alfalfa virus 2*.

The writer has made no tests on the seed transmission of tobacco ring-spot virus nor of *tobacco virus 1*. Transmission of a ring spot of tobacco through the seed of tobacco in a few instances was reported by Johnson (20). Valleau[3] reported that evidence had been obtained to show that tobacco ring spot was transmitted through tobacco seed. Henderson (16), although unable to confirm the report of Valleau, demonstrated that ring spot was transmitted through the seed of garden petunia to about 20 per cent of the seedlings. To the writer's knowledge no proof of transmission of *tobacco virus 1* through the seed of tobacco has been presented.

Host Range

Each of the 5 viruses considered was tested on the following species of Leguminosae and Solanaceae: hyacinth bean, *Dolichos lablab* L.; lespedeza, *Lespedeza striata* Hook and Arn.; white lupine, *Lupinus albus* L.; common and Turkestan alfalfa, *Medicago sativa* L.; white sweet clover, *Melilotus alba* Desr.; black tepary bean, *Phaseolus acutifolius* Gray var. *latifolius* Freem.; adzuki bean, *Phaseolus angularis* Wight; mung bean, *P. aureus* Roxb.; rice bean, *P. calcaratus* Roxb.; scarlet runner bean, *P. coccineus* L.; Lima bean, Burpee's bush variety, *P. limensis* Macf.; Sieva Lima bean,

[3] Valleau, W. D. Tobacco seed beds in Kentucky. U. S. Dept. Agr. Pl. Dis. Rptr. 14: 113. 1930. (Mimeogr.)

Henderson bush variety, *P. lunatus* L.; garden pea, Perfection variety, *Pisum sativum* L.; kudzu bean, *Pueraria hirsuta* Schneid.; soybean, Manchu and Mikado varieties, *Soja max* Piper; velvet bean, *Stizolobium* sp.; alsike clover, *Trifolium hybridum* L.; crimson clover, *T. incarnatum* L.; red clover, *T. pratense* L.; white clover, *T. repens* L.; broad bean, *Vicia faba* L. var. *minor;* asparagus bean, *Vigna sesquipedalis* Wight; cowpea, Groit variety, *V. sinensis* Endl.; tomato, Globe variety, *Lycopersicon esculentum* Mill.; tobacco, *Nicotiana tabacum* L. and *N. glutinosa* L. and garden petunia, Star of California variety, *Petunia hybrida* Vilm. The number of plants inoculated in each case varied from 5 to 25 or more. All inoculations were made artificially by the wiping method. Infection in hosts reported as susceptible was confirmed by inoculations to Refugee Green bean. By this procedure the presence or absence of any particular virus was ascertained, even though the inoculated plants did not show visible symptoms.

The reports of Reddick and Stewart (31) that *Phaseolus lunatus* var. *macrocarpus, P. acutifolius* var. *latifolius,* and *Vicia faba* and of Nelson (26), that *Phaseolus calcaratus* Roxb. and *P. lunatus* L. were susceptible to common bean mosaic were confirmed. Besides these hosts the mung bean, lespedeza, and spring vetch were found to be susceptible. Repeated inoculations with *bean virus 1* to Henderson Bush Lima bean gave only a small percentage of infection; it appears, therefore, that this variety is quite resistant to infection by this virus. The writer has been unable to confirm Nelson's (26) report that *Phaseolus angularis, P. coccineus,* and *Vigna sesquipedalis* are susceptible to common bean mosaic.

The host range of yellow bean mosaic (*bean virus 2*) parallels rather closely that of common bean mosaic. With the exception of Burpee's Bush Lima, *Phaseolus limensis,* Henderson Bush Lima, *P. lunatus,* and rice bean, *P. calcaratus,* all of the hosts of the latter were found to be susceptible to yellow bean mosaic. Hosts found to be susceptible to yellow bean mosaic and not to common bean mosaic were white sweet clover, soybean, white lupine, and crimson clover. Juice extractions of certain naturally infected sweet clover and soybean plants have been tested on bean and found to produce symptoms typical of yellow bean mosaic. In other transfers from mosaic sweet clover to bean, however, no infection of any kind was obtained, indicating that some virus not considered in this paper was concerned.

The host range of *alfalfa virus 2* is distinctly wider than that of the two bean viruses, as infection was obtained on certain solanaceous hosts as well as on a large number of leguminous hosts. In addition to certain varieties of common bean, the asparagus bean was found to develop local lesions after inoculation. Systemic infection was secured upon the follow-

ing: hyacinth bean, adzuki bean, mung bean, rice bean, common and Turkestan alfalfa, white sweet clover, crimson clover, red clover, white clover, Perfection pea, broad bean, spring vetch, Manchu and Mikado soybeans, garden petunia, and tobacco, *Nicotiana tabacum* and *N. glutinosa.*

It is beyond the scope of this paper to describe in detail the symptoms on all the hosts infected with different viruses. However, it should be mentioned that certain very definite differences were often apparent. For instance, soybeans infected with *alfalfa virus 2* exhibit certain characteristic symptoms differing in many respects from symptoms on soybeans infected with *bean virus 2.* Another example is that of broad bean, *Vicia faba,* on which infection with *alfalfa virus 2* results in a very pronounced vascular necrosis, finally killing the plants, while mottling of the leaves is the conspicuous symptom produced by *bean virus 2.*

Infection with *tobacco virus 1* was not secured on any of the legumes tested, except local infection upon certain varieties of *Phaseolus vulgaris.* The solanaceous hosts tested were all previously recognized hosts of this virus.

Tobacco ring-spot virus was found to infect a wide range of hosts. Priode (29) transferred ring spot to beet, *Beta vulgaris* L.; pokeweed, *Phytolacca decandra* L.; petunia, *Petunia hybrida,* and New Zealand spinach, *Tetragonia expansa* Murr. In a study of the host range of tobacco ring spot, Wingard (35) obtained successful transmission to 38 genera of plants, including transmission to the following Leguminosae: *Dolichos lablab, Melilotus officinalis* Lam., *Phaseolus lunatus, P. vulgaris* L., and *Vigna sinensis.* In the present study several other leguminous plants were found to be susceptible. The ring-spot virus produced only local necrotic lesions on the inoculated leaves of the following hosts: Henderson Bush Lima, *Phaseolus lunatus,* Burpee's Bush Lima, *P. limensis,* and for the most part of the varieties Corbett Refugee, Robust, and Mexican Tree of *Phaseolus vulgaris.* On many hosts local necrotic infection is followed by systemic infection, which in nearly all cases causes the death of the plants involved. Hosts thus affected were white lupine, Groit cowpeas, asparagus bean, soybean, broad bean, spring vetch, velvet bean, mung bean, rice bean, and many varieties of common bean.

The following hosts were found to develop systemic symptoms and did not develop visible local necrotic lesions at the points of inoculation: hyacinth bean, white sweet clover, crimson clover, and adzuki bean.

It is apparent that the host range of each of the five viruses here considered is different. A few of the more outstanding differences which may be useful in differentiating between them have been summarized in table 6.

Separation of the Viruses Studied

The technique of separation of two or more viruses present in a single plant, on the basis of their host range and properties has been described by Johnson (21). The same principles may be employed in the separation of the 5 viruses considered in this paper. A summarized scheme for their differentiation and identification on the basis of hosts is presented in table 6.

The isolation of *bean virus 1* from mixtures with the other viruses is probably the most difficult to accomplish, since it is very sensitive to treatments of any kind. It may be separated from the others through the medium of the seed. By inoculating any virus mixture containing *bean virus 1* to the Mexican Tree variety, this virus would produce systemic infection, while *alfalfa virus 2, tobacco virus 1,* and tobacco ring-spot virus cause local infection. If *bean virus 2* were not present in the mixture *bean virus 1* could be obtained from the new growth showing mosaic symptoms. If *bean virus 2* were present it would be necessary to grow the plants to maturity and obtain *bean virus 1* from the seed.

Bean virus 2 may be secured from a mixture with any of the other viruses by inoculation to Corbett Refugee, this variety being resistant to systemic infection by all the other viruses.

Alfalfa virus 2 may be isolated from the other four viruses by inoculation to Perfection variety of pea, which is susceptible to the alfalfa virus but not to the other viruses. In case of a mixture with the 2 bean viruses, *alfalfa virus 2* may be separated from them by aging a mixed sample of the viruses for a period of 2 or 3 days. This length of time insures the inactivation of the 2 bean viruses but not that of the alfalfa virus.

The tobacco ring-spot virus may be secured free from any of the other viruses considered here by inoculation of cowpeas (Groit variety). Another method is to treat the virus mixture with 75 per cent alcohol for 30 minutes or with heat at 64° C. for 10 minutes. This inactivates all viruses except ring-spot and *tobacco virus 1.* The latter can be eliminated by inoculation of a variety of *Phaseolus vulgaris* resistant to it, such as Red Valentine.

Tobacco virus 1 in combination with any of the above viruses may be secured by inoculation of tomato, or by sufficient heating or aging to inactivate the other viruses in the mixture.

DISCUSSION

The significance of the investigations presented in this paper is largely in the accumulation of evidence that bean and other legumes may be subject to a number of different viruses, and that these viruses may be readily differentiated or separated in various ways. It also is hoped that the description of certain new or previously undescribed viruses will be sufficiently

TABLE 6.—*Summarized scheme for the differentiation and identification of bean virus 1, bean virus 2, alfalfa virus 2, tobacco virus 1, and tobacco ring-spot virus on basis of differential hosts*[a]

Virus	Type of infection	HOSTS								
		Phaseolus vulgaris					*Pisum sativum*	*Vigna sinensis*	*Nicotiana tabacum*	*Nicotiana glutinosa*
		Refugee Green	Corbett Refugee	Mexican Tree	Robust	Red Valentine	Perfection pea	Groit cowpeas	Tobacco	
Bean virus 1	Local	—	—	—	—	—	—	—	—	—
	Systemic	++	—	++	—	++	—	—	—	—
Bean virus 2	Local	—	—	—	—	—	—	—	—	—
	Systemic	++	+	++	+	++	—	—	—	—
Alfalfa virus 2	Local	++	±	±	±	++	—	—	—	—
	Systemic	—	—	—	—	—	+	—	±	±
Tobacco virus 1	Local	+	+	++	++	—	—	—	—	++
	Systemic	—	—	—	—	—	—	—	++	—
Ring-spot virus	Local	±	±	+	+	++	—	+	+	—
	Systemic	++	—	±	±	++	—	+	+	+

[a] — indicates no infection; ± slight infection; + moderate infection; ++ severe infection.

complete to permit their definite recognition by future workers with legume viruses.

For purposes of preliminary comparison we have included in this study 3 viruses (*alfalfa virus 2, tobacco virus 1,* and the tobacco ring-spot virus), which are not known to affect the bean in nature. This fact should not be allowed to confuse the general understanding of the bean-virus problem itself. Besides the common bean-mosaic virus (*bean virus 1*) we have shown that another virosis of the bean occurs in nature, which has been named yellow bean mosaic (*bean virus 2*). That still other viruses may affect the bean is evident from Carsner's (6) transmission of the sugar beet curly-top virus to this host. Beyond this, however, the distinction between the above mentioned viruses and others reported to occur on bean and other Leguminosae becomes less apparent. Nelson's (26) rugose mosaic of the bean appears on the basis of transmission through the seed to be distinct from yellow bean mosaic, which it, symptomatically, most resembles.

The possible relationships and overlapping host ranges of bean viruses to those of other leguminous plants may be illustrated by the following cases. Transmission experiments with aphids led Merkel (24) to the conclusion that one and the same virus was responsible for the mosaic symptoms of all the Papilionaceae that he examined, which included *Phaseolus vulgaris, Pisum sativum, Lathyrus odoratus, Lupinus luteus, Melilotus altissima, Trifolium pratense, T. hybridum, T. repens, Anthyllis vulneraria,* and *Vicia faba.* Zaumeyer (36) transmitted to beans the viruses of mosaics of *Melilotus alba, M. officinalis, Trifolium repens, T. hybridum, Pisum sativum,* and *Lathyrus odoratus.* He stated that the virus of mosaic white sweet clover produced symptoms on beans slightly different from those of common bean mosaic. The virus concerned may have been identical with *bean virus 2* described in this paper.

There are, on the other hand, a number of reports of mosaics on leguminous plants that have not been connected with viruses affecting the common bean. Doolittle and Jones (8) described a garden-pea mosaic transmissible to sweet pea and red clover. They were, however, unsuccessful in producing the disease by inoculations from mosaic sweet clover, *Melilotus alba,* and mosaic bean, *Phaseolus vulgaris,* to garden peas and sweet peas. Böning (5) considered a virus disease of broad bean, which he found transmissible to red clover, crimson clover, and peas, as probably identical with the pea mosaic of Doolittle and Jones (8). The same virus may also have been concerned in the mosaic of *Trifolium pratense,* which Dickson (7) had previously shown to be transmissible to several species of Trifolium and to *Medicago lupulina* but not to *Melilotus alba* nor *M. officinalis.* Elliott (9), on the other hand, may have been concerned with another virus in his mosaic of sweet clover and red clover, since he found the virus to be transmis-

sible to the two latter hosts as well as to *Medicago arabica* and *Vicia faba*, but not to *Trifolium repens* nor *Medicago sativa*. As previously pointed out, the soybean mosaic of Kendrick and Gardner (23) was shown by them to be nontransmissible to bean; it would appear, therefore, that a specific virus, differing from any described in this paper, was responsible for their soybean mosaic.

It seems to be sufficiently evident from these investigations that further progress in and understanding of the viroses of the legumes as a whole or the virosis of any species in particular are dependent upon a more comprehensive study of the viruses themselves rather than of the diseases caused by them.

The existence in the bean of distinct immunity from and susceptibility to the common bean mosaic affords a promising field for the investigation of the inheritance of resistance to this disease, as well as for the development of new resistant commercial varieties. The fact that more than one mosaic disease exists necessarily complicates such a problem. Since no varieties of the common bean have been found that are entirely resistant to yellow bean mosaic, a continuation of the development of beans immune from common bean mosaic and moderately resistant to yellow bean mosaic would appear at present to offer the most promising means of eliminating losses from both diseases. It has been demonstrated that it is possible to secure families resistant to common bean mosaic from crosses of Corbett Refugee and Refugee Green. It is probable, therefore, that Corbett Refugee will be useful as a resistant parent variety for the improvement of many susceptible varieties of the canning type.

Since yellow bean mosaic (*bean virus 2*) is either not seed borne in the bean, or, if so, to only a slight extent, its chances of becoming of serious economic importance are greatly reduced. The fact that sweet clover has been found to be a host of *bean virus 2* suggests a means by which the virus probably overwinters. Isolation of bean plantings from infected sweet clover may prove effective in the control of yellow bean mosaic.

SUMMARY

The studies reported in this paper were concerned primarily with the resistance of varieties of *Phaseolus vulgaris* to the virus of common bean mosaic. In the course of the study other distinct viruses were found to affect the bean. To allow for the specific naming of such of them as had not been previously named an attempt has been made to differentiate the viruses on the basis of symptom expression, varietal susceptibility, properties *in vitro*, and transmissibility through the seed.

The susceptibility of 24 varieties of beans to the following viruses was determined by means of artificial inoculations in the greenhouse: common bean-mosaic virus (*bean virus 1*), yellow bean-mosaic virus (*bean virus 2*),

alfalfa-mosaic virus (*alfalfa virus 2*), tobacco-mosaic virus (*tobacco virus 1*), and tobacco ring-spot virus. *Bean virus 2* and *alfalfa virus 2* had not been previously described.

Bean virus 1 and *bean virus 2* both produced systemic infection on beans, but the symptoms produced by *bean virus 2* were much more severe. *Alfalfa virus 2* and *tobacco virus 1* caused only local necrotic infection; and the tobacco ring-spot virus caused both local and systemic infection on beans. Symptoms on differential varieties were described.

The bean varieties were grouped according to symptom expression and susceptibility to *bean virus 1* as either: I, susceptible; II, tolerant; or III, immune. In general the same grouping held for reaction to *bean virus 2,* although none of the varieties were found to be immune from this virus. The susceptibility to the 2 bean viruses was not correlated with susceptibility to the other viruses studied.

The thermal inactivation point, resistance to aging *in vitro,* tolerance to dilution, and resistance to certain chemicals were determined for all the viruses except *tobacco virus 1;* the properties of the latter have been previously determined by Allard and others. The thermal death point of *bean virus 1* and *bean virus 2* was found to lie between 56° and 58° C. for 10 minutes; of *alfalfa virus 2* between 62° and 64° C.; and of tobacco ring-spot virus at 66° C. In aging-*in-vitro* experiments, *bean virus 1* and *bean virus 2* lost their infectivity after 24 to 32 hours; *alfalfa virus 2* and tobacco ring-spot virus in from 7 to 9 days. The infectivity of all the viruses was either lost or greatly reduced at dilutions greater than 1–1000. Treatments with alcohol, nitric acid, and formaldehyde showed minor differences in the resistances of the viruses to these treatments.

Previous reports of transmission of common bean-mosaic virus by the potato aphid, *Macrosiphum solanifolii,* and by the pea aphid, *Illinoia pisi,* were confirmed. *Bean virus 2* was also shown to be transmitted by these aphids. *Alfalfa virus 2* was readily transmitted by the pea aphid.

In tests involving 6532 seedlings no evidence of transmission of *bean virus 2* through the seed was obtained. *Bean virus 1* was transmitted through the seed, as previously reported.

The host-range studies showed important differences between the viruses studied. Sweet clover was found to be susceptible to *bean virus 2,* and it was suggested that this host may serve to overwinter this virus.

While specific viruses other than those described in this paper play a part in the virus infections occurring on leguminous plants, it is hoped that the data presented in this paper will be of value in the ultimate differentiation of all the viruses affecting plants of the Leguminosae.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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RESISTANCE OF POTATO TO MOSAIC AND OTHER VIRUS DISEASES[1]

E. S. Schultz,[2] C. F. Clark,[3] Reiner Bonde,[4] W. P. Raleigh,[5] and F. J. Stevenson[6]

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INTRODUCTION

The use of resistant varieties of plants is the most effective method of disease control. Certain potato diseases, such as late blight, can be controlled to some extent by spraying but in years of severe epidemics they cause heavy losses in spite of control measures. Other potato diseases can be partially controlled by seed treatment and by soil disinfection. Potato viroses, however, cannot be controlled by these methods.

At present the most effective methods for the control of a potato virosis are tuber indexing, early and thorough roguing, and early harvesting of isolated tuber-unit seed plots, together with the importation of seed from a region in which spread from plant to plant is generally infrequent, or the use of an immune, resistant, or tolerant variety. The last method of control has been applied to mild mosaic in commercial practice to the extent that the resistant Irish Cobbler and Spaulding No. 4 (Syn. Rose No. 4) varieties are grown. Proof of this resistance and the development of other resistant varieties are described in this paper, together with experiments with other potato viroses.

DESCRIPTION OF DISEASES

Mild mosaic is manifested on the variety Green Mountain by slight wrinkling, ruffling, and dwarfing and by distinctly light green spots more or less indistinctly delimited by irregular margins from the dark green leaf areas. Next to latent mosaic, or seedling streak, mild mosaic is found most commonly in Green Mountain in the important seed-potato localities.

Leaf roll is manifested by rolling and rigidity of the leaves and by distinct dwarfing and chlorosis. Tubers of leaf-roll plants usually are sessile and reduced in size and number.

Spindle tuber is manifested by more or less spindling, erect, slightly rugose plants, which are dwarfed and somewhat darker green than healthy tops. Spindle-shape tubers also distinguish this malady from other viroses.

[1] Conducted as a cooperative project between the Bureau of Plant Industry, U. S. Department of Agriculture, and the Maine Agricultural Experiment Station.

[2] Senior Pathologist, U. S. Department of Agriculture.

[3] Associate Horticulturist, U. S. Department of Agriculture.

[4] Associate Plant Pathologist, Maine Agricultural Experiment Station.

[5] Associate Pathologist, U. S. Department of Agriculture.

[6] Geneticist, U. S. Department of Agriculture.

Although our observations were concerned primarily with resistance of potato varieties and seedlings to mild mosaic, leaf roll, and spindle tuber, less extensive studies also were made on resistance of seedlings to streak, which also has been called stipple streak, or acropetal necrosis, and to seedling streak, or top-necrosis.

Streak is characterized on Green Mountain in current season infections by necrotic streaking and spotting on leaves, petioles, and stems and by brittleness, leaf fall, and premature death. Second-season symptoms are manifested by severe dwarfing, necrotic spotting and streaking, and premature death, which usually precedes tuber formation.

Top-necrosis, or acronecrosis (Quanjer 10), frequently has been designated as seedling streak in our work, since it was encountered in connection with studies on the reaction of different potato seedlings to mosaic and other viroses. Inasmuch as Green Mountain was found to be a latent carrier of top-necrosis, this disease also has been called latent mosaic. As the name of this disease indicates, it involves at first the apical part of the plant where it causes severe necrosis on leaf, petiole, and stem. Necrosis appears in cortex and pith of stems, as well as in tubers. Eyes of tubers may be killed or partly infected so that necrotic as well as apparently healthy shoots may develop from such tubers during the season that follows the one in which infection occurred. Acronecrosis is not generally so superficial as streak, or acropetal necrosis.

METHODS OF INOCULATION USED IN TESTING PLANTS FOR RESISTANCE

Plants were exposed to infection by two methods: (1) Healthy plants were planted either in hills alternating with hills of diseased plants or in rows alternating with rows of diseased plants. (2) Healthy tubers were grafted with diseased tubers by means of splice or plug grafts. The Green Mountain variety served as the source for diseased plants and as a healthy control. Since symptoms of infection are not always discernible in the season in which the infection takes place, the tubers from the exposed plants were grown the next season and disease data secured.

In the field tests during 1924 to 1930 25 hills of each variety or seedling to be tested were planted alternately with hills of diseased material. Three series were grown, 1 for each of the 3 diseases, mild mosaic, leaf roll, and spindle tuber.

In 1931, the seedlings were planted in 10-hill lots, except in a few lots of the 2 naturally fertilized progenies where sufficient seed stock was not available for 10 hills. These lots were planted consecutively in rows alternating with Green Mountain potatoes affected with mild mosaic. Ten-hill lots of apparently healthy Green Mountain seed pieces were planted as controls in representative parts of each seedling row.

Most of these experiments were conducted at Presque Isle, Maine, where conditions usually favor the expression of mosaic symptoms. Some of the tuber grafts were made and planted in the greenhouse at Arlington Farm, Virginia.

Field Exposure of Potatoes to Mild Mosaic, Leaf Roll, and Spindle Tuber, 1924–1930. Thirty-three potato seedlings representing 18 crosses were exposed to Green Mountain potatoes affected with mild mosaic under field conditions in northern Maine in 1924–1930. During the first 4 years of these tests, 1924–1927, 2 tubers per hill were saved for the following season's planting, but from 1928 to 1930, inclusive, 3 tubers were harvested for this purpose.

Tuber-graft Inoculations. In the tuber-graft inoculations usually 8 to 10 tubers of a variety or seedling were plug- or splice-grafted with diseased tubers. A seed piece of each of the healthy and the diseased tubers was planted as a control. In most of these inoculations the Green Mountain variety represented the source of the diseased material.

Johnson (4) and Schultz (12) reported that certain potato varieties, like the Green Mountain, were susceptible to mild mosaic and at the same time were masked carriers of latent mosaic, resulting in mixed or composite infection. In the early years of the experiments here reported seedlings were produced by hybridization and selection, some of which were susceptible to both mild and latent mosaic, giving the same complex as that found in Green Mountain, others that were somewhat susceptible to the mild but resistant to the latent form, and still others that were resistant to the mild mosaic but susceptible to the latent. In this last group the latent form often expresses itself as severe streak or necrosis. The seedlings that are resistant to latent mosaic simplify the work since they make it possible to separate the mild form from the latent type. Accordingly, in 1931 and 1932, a seedling that becomes infected with mild mosaic but is resistant to latent mosaic was used instead of Green Mountain as the source of mild mosaic in the tuber-graft tests.

REACTION OF POTATO TO LEAF ROLL AND SPINDLE TUBER

From 1924 to 1930, 33 potato seedlings and Green Mountain, Irish Cobbler, and Spaulding No. 4 were exposed to spindle tuber. In 1924, 1925, 1928, 1929, and 1930, 18 potato seedlings also were exposed to leaf roll, under the same method used for exposing these potatoes to mild mosaic. The results recorded in table 1 disclose that nearly every seedling and variety became infected with these diseases. No apparent differences in severity of symptoms of leaf roll and spindle tuber were observed between these selections. However, some of the seedlings manifested spindle tuber on the tubers in the form of pear-shape tubers instead of spindle-shape

tubers, as commonly found in Green Mountain. A few seedlings apparently reacted to spindle tuber by manifesting slightly rolled leaves in addition to the erect and more or less spindling tops, which are characteristic symptoms of spindle tuber.

Leaf roll and spindle tuber spread much more generally in 1929 than during the other seasons. Inasmuch as most of the seedling lots were infected with spindle tuber when they were introduced to the trial plots, the percentage of spindle tuber recorded in table 1 represents only a part of the infection contracted in the exposure tests. Since spindle tuber is spread by means of the cutting knife, it is possible that this method of infection also contributed to the results.

Experience with leaf roll and spindle tuber has shown that these maladies usually are spread less widely in northern Maine than mild mosaic. Consequently, field exposure tests with mild mosaic in this locality have been more reliable than similar trials with leaf roll and spindle tuber.

REACTION OF POTATO TO STREAK

Observations on the reaction of Spaulding No. 4 and Green Mountain to streak have disclosed that Spaulding No. 4 manifests the symptoms of this disease in milder form than Green Mountain. Although Spaulding No. 4 develops necrotic spots and streaks on leaves, stems, and petioles, these symptoms are not so conspicuous as those on Green Mountain. Spaulding No. 4, affected with streak, produces a fair crop of tubers, while streak-affected Green Mountain tops from streak-infected tubers are so severely dwarfed, streaked, and curled that tuber development usually is inhibited.

In 1931, tubers from several apparently healthy potato varieties were tuber-grafted on apparently healthy Green Mountain tubers. The Green Mountains tuber-grafted to one of these varieties developed typical symptoms of streak, which indicates that this variety is a latent or masked carrier of this disease. Atanasoff (1) pointed out that he found certain varieties that were masked carriers of streak.

REACTION OF POTATO TO LATENT MOSAIC

The term latent mosaic is not used here to designate a single virus but it may include several viruses the symptoms of which are not manifested in some varieties. It is harbored in masked or latent form by Green Mountain, Irish Cobbler, Bliss Triumph, and other commercial varieties. Some of these varieties or strains of them have very few plants that are free from latent mosaic.

Among the commercial varieties tested none has been found that does not carry latent mosaic in one form or another in a high percentage of plants. A seedling, U. S. D. A. number 41956, has been produced, however,

that has shown resistance to latent mosaic. Leaf rubbing and tuber-graft inoculations with this virus on this seedling, which has been tested for several years, have so far failed to induce infection. Comparable inoculations on Green Mountain seedlings free from latent mosaic resulted in every plant becoming infected with this disease.

In field tests seedling 41956 has been grown for several seasons in contact with latent-mosaic Green Mountain but has not contracted the disease in a single case. On the other hand, latent-mosaic-free Green Mountain seedlings, similarly propagated, contracted latent mosaic in 15 per cent of the plants in a single season. Since leaf-rubbing, tuber-grafting, and field-contact tests have failed to produce infection of latent mosaic in seedling 41956, it is highly resistant to this virus under all conditions involved in the tests. Schultz and Raleigh (14).

Graft and juice inoculations from masked carriers of latent mosaic to certain other varieties or seedlings result in severe necrosis on tops and tubers. This necrosis has been designated top-necrosis or acronecrosis. (Quanjer 10). Seedlings showing this reaction have been found that seldom contract infection when exposed to latent-mosaic Green Mountain in the field. If these are compared with the Green Mountain seedlings noted above, which contracted latent mosaic in 15 per cent of the plants in a single season, they can be considered resistant to latent mosaic in field tests. Under the more severe tests of tuber grafting and juice inoculation, top necrosis developed. While it is highly desirable to produce varieties resistant to this or any disease, under all conditions, it is, nevertheless, important from the standpoint of breeding commercial varieties to produce seedlings resistant to a disease when grown under the usual field conditions. As the vector for latent mosaic has not been found, it is impossible to specify the factors that may be responsible for the differences in the response of these seedlings to latent mosaic under these conditions. However, since latent mosaic can easily be transmitted by leaf rubbing, it appears very possible that contact of diseased with healthy plants in the field may transmit latent mosaic and that such transmission may occur more readily in some varieties than in others. In connection with their investigations on necrotic viroses of potato, Salaman and Bawden (11) point out that the evidence is suggestive of the existence of a definite resistance to certain viruses in the variety Abundance. Among these viruses was included a virus or virus complex that apparently is identical with the one involved in latent mosaic.

REACTION OF POTATO TO MILD MOSAIC

Experience with Irish Cobbler has shown that this variety apparently fails to react to mild mosaic. Leaf-rubbing and tuber-graft inoculations from mild-mosaic Green Mountain to Irish Cobbler have apparently failed

to infect this variety. Likewise, return or back grafting of tubers from Irish Cobblers inoculated with mild mosaic to apparently healthy Green Mountains has failed to produce infection. Inasmuch as more than a hundred such tuber grafts have been made at various times and planted in field and under greenhouse conditions without producing mild mosaic on Irish Cobbler, and since this variety has failed to manifest symptoms of mild mosaic after having been grown for several seasons next to mild-mosaic Green Mountain, it is without doubt resistant to mild mosaic. However, Irish Cobbler, like Green Mountain, is a symptomless carrier of latent mosaic and clearly manifests the same reactions to rugose mosaic, acropetal necrosis, and other potato viroses, as does Green Mountain.

Our experience indicating that the Irish Cobbler variety manifests resistance to mild mosaic has been confirmed by seed-potato growers who find that leaf roll, spindle tuber, and rugose mosaic are the diseases they must eliminate from this variety in order to maintain it free from viroses. Growers frequently have propagated Irish Cobbler adjoining mild-mosaic Green Mountain, with the result that the Irish Cobbler failed to contract mild mosaic, while healthy Green Mountain similarly propagated developed more than 50 per cent of this disease during a single season.

The differences observed between varieties and seedlings with respect to their reaction to mild mosaic were the basis of a systematic breeding program, which was started for the purpose of producing new seedling varieties in which it was sought to combine resistance to this disease with other desirable commercial qualities. Careful observations were made of the most fertile seedlings and data secured on the amount of mosaic found when grown under the conditions prevalent in the plant-breeding plots for a series of years. Two of these, S. 24642 and S. 40568, were comparatively free from mild mosaic for a number of years. For the 3 years 1918, 1920, and 1921, S. 24642 was free from mild mosaic, and in 1919 it showed approximately 2 per cent. For the same period of time, 1918–1921, S. 40568 produced one mild-mosaic plant out of approximately 500 plants grown. In comparison with this, S. 39477, one of the susceptible seedlings, produced no mild mosaic in 1919, 2.7 per cent in 1920, and 49.7 per cent in 1921. From the data available in 1921, then, S. 24642 and S. 40568 were apparently resistant to mild mosaic. Consequently a large number of crosses were made in which these seedlings were used as parents. These 2 seedlings were crossed with each other and with other seedlings and varieties.

As the main object of the early breeding work was to obtain varieties superior in many respects to the commercial varieties only those seedlings were selected from the progenies of these crosses that were desirable from the standpoint of shape, shallowness of eye, yield, and other characters of economic importance. In the cross between the 2 seedlings S. 24642 and

S. 40568, 40 selections were made in 1923. A number of these were found to be more susceptible than either of the parents under field conditions, since 6 of them developed 100 per cent mild mosaic in one year, 1924. On the other hand, 2 selections of this progeny, S. 42667 and S. 42672, grown under the same conditions, have shown a higher degree of resistance than either of their parents. These 2 seedlings and 31 others, representing in

TABLE 1.—*Reaction of certain potato varieties to viroses. Potatoes exposed to infection in the field, Aroostook Farm, Presque Isle, Maine—1924–1930*

Variety	Year	Total hills	Symptoms and hills infected					
			Mild mosaic		Leaf roll		Spindle tuber	
			No.	Per cent	No.	Per cent	No.	Per cent
Green Mountain	1924	50	20	40			5	10
	1925	50	22	44	5	10	3	6
	1926	50	42	84			2	4
	1927	50	23	46			3	6
	1928	75	68	91	4	5	1	1
	1929	75	72	96	46	61	46	61
	1930	75	29	39	2	2	10	14
Irish Cobbler	1924	50					22	44
	1925	50			3	6	2	4
	1926	50					5	10
	1927	50					3	6
	1928	75					6	8
	1929	75			50	66	48	64
	1930	72					15	20
Spaulding No. 4	1924	50					14	28
	1925	50			10	20	8	16
	1926	50					2	4
	1927	50					2	4
	1928	75			5	7		
	1929	75			38	51	47	63
	1930	75					48	66
Jersey Red Skin	1926	50	23	46			11	22
Early Ohio	1925	50	8	16	7	14	23	46
24642	1924	50	13	26	2	4	8	16
	1925	50	5	10			5	10
41420	1925	50	4	8	14	28	12	24
41499	1926	50	4	8			20	40
41558	1925	50	2	4			3	6
41803	1925	50	8	16	4	8	40	80

TABLE 1.—(*Continued*)

Variety	Year	Total hills	Symptoms and hills infected					
			Mild mosaic		Leaf roll		Spindle tuber	
			No.	Per cent	No.	Per cent	No.	Per cent
41865	1925	50	7	14			21	42
41881	1926	50	4	8			11	22
41914	1924	50	10	20			12	24
41914	1930	75					14	18
41916	1924	50	8	16			13	26
41917	1924	50	2(?)	4	11	22	6	12
41918	1926	50	7	14			4	8
41956	1925	50	3	6	4	8	25	50
41956	1927	50	7	14				
41956	1930	75	1	1	4	5	13	17
41977	1925	50	12	22			10	20
41988	1926	50	13	26			11	22
42016	1926	50	12	22			3	6
42667	1929	75			22	29	30	40
42667	1930	75					18	24
42672	1929	75			67	89	66	88
42672	1930	75			6	8	27	36
42857	1927	50	3	6				
42857	1928	75					9	12
42858	1928	75	4	5	6	8		
42898	1927	50	4	8			10	20
42901	1928	75	1	1	1	1		
42903	1928	75	12	16	9	12	3	4
42906	1927	50	10	20			2	4
42949	1927	50	5	10			8	16
42953	1927	50	7	14			3	6
42963	1928	75	1	1	10	13	13	17
42966	1928	75	4	5	4	5	9	12
42967	1928	75	1	1	5	7		
43011	1927	50	9	18			2	4
43106	1927	50	3	6			4	8
43313	1928	75	1	1	12	16	9	12
43325	1928	75	20	27	10	13		
43327	1928	75	12	16	25	33		

all 18 different crosses, were subjected to more severe tests than those that prevailed in the plant-breeding plots. These 33 seedlings were grown in proximity to Green Mountain plants infected with mild mosaic. These tests extended over the period of years from 1924 to 1930, inclusive. Six of these were exposed during 2 or more seasons, but the remaining 27 were exposed during one season only. Table 1 gives the data for these tests.

The two resistant seedlings, S. 42667 and S. 42672, failed to develop mild mosaic in this test, although their parent, S. 24642,[7] gave an average of 18 per cent for the two years 1924 and 1925. The percentage of mosaic in the remaining 30 seedlings varied from 1 to 27. Most of these contracted less than 10 per cent mosaic, while the Green Mountain checks developed from 39 to 96 per cent of mild mosaic. A few of the seedlings manifested symptoms of mild mosaic like those in Green Mountain, but in most of them the presence of this disease was indicated by faint mottling and slight wrinkling or rugosity or by symptoms that are less pronounced than those in mild-mosaic Green Mountain. It is possible that these faint mosaic symptoms may be due partly to the absence of latent mosaic. On the other hand, these faint mosaic symptoms may be the expression of mild mosaic in a seedling that is partly resistant to the virus. None of the potato seedlings in the test reported here developed mosaic in as many plants as the Green Mountain controls, and only a few of them manifested symptoms of mild mosaic like those in Green Mountain. It should be pointed out here that the seedlings reported in table 1 were the apparently resistant selections made in the plant-breeding plots and that many of the sibs of these seedlings were extremely susceptible, some of them producing 100 per cent mosaic plants in the plant-breeding plots in the second year after being grown from true seed. Seedling 42667, a selection from the progeny of the cross between the two resistant seedlings 24642 and 40568, is very highly resistant to mild mosaic. It has been grown in the plant-breeding plots for 10 years entirely free from this disease. During the same time many other seedlings, grown under the same conditions, produced 100 per cent mild mosaic. It remained free from mild mosaic after it had been grown adjoining mild-mosaic Green Mountain in the disease test plots for 3 seasons. During this period Green Mountain controls carrying latent mosaic developed mild mosaic in 80 per cent of the plants. Seedling 42667 has been subjected to the more severe tests of tuber grafting, with results that again show a high degree of resistance to mild mosaic.

In 1930 this seedling was tuber-grafted on mild-mosaic Green Mountain. Splice and plug grafts were made. In the plug grafts 2 plugs of a diseased tuber were inserted into the healthy tuber, except in series 6, table 2, in which healthy plugs were inserted into the diseased tuber from which the eyes had been removed. The healthy as well as the mild-mosaic Green Mountain carried latent mosaic. Since S. 42667, grafted on latent-mosaic Green Mountain, developed top necrosis, as shown in series 1, later grafts were made on mild-mosaic seedling 41956, which did not carry latent mosaic. The results of these grafts are recorded in table 2.

[7] In the breeding plots that represent ordinary field conditions the average per cent of mild mosaic found in this seedling for a period of 12 years was 1.4.

TABLE 2.—*Reaction of seedling 42667 to mild mosaic. Tuber-graft inoculations, Presque Isle, Maine, 1930–1932*

Series	Graft	Number plants infected / Number tubers grafted[a]	Symptoms
1	S. 42667 on mild-mosaic G. M.[b]	3/8	Tip necrosis on S. 42667
2	Healthy G. M. on S. 42667 showing top necrosis in Series 1	1/9	Mosaic type? G. M. Mottled (mosaic type?)[c] nongraft control S. 42667
3	Healthy G. M. on S. 42667 showing few mottled areas	0/5	
4	S. 42667 on mild-mosaic 41956	0/12	
5	Do	0/20	
6	S. 42667 plugs inserted into mild-mosaic 41956 tubers	2/10	Light-green patches on S. 42667 (mosaic type?)
7	Healthy G. M. on mild-mosaic 41956	9/20	Mild-mosaic G. M.
8	Healthy G. M. on S. 42667 exposed to mild-mosaic G. M. in field during 2 seasons	0/100	

[a] The numerator indicates the number of plants infected, and the denominator the number of tubers grafted.

[b] G. M.—Green Mountain.

[c] Inoculations with this type of mosaic made by the writers after the manuscript of this paper was submitted indicate that this mosaic type may be the same as latent mosaic.

Since top necrosis was the only reaction maninfested by S. 42667 in series 1, the tuber progeny of the 3 hills showing top necrosis were reserved for back or return grafts on healthy Green Mountain. The results of these back grafts are recorded in series 2, which shows that one of the 9 grafted tubers developed mosaic. The S. 42667 plants from the nongrafted control tubers in series 2 manifested slightly rugose leaves with distinctly mottled spots. Since additional tuber grafts with these mottled S. 42667 on healthy Green Mountains, as indicated in series 3, yielded no apparent infection, it is not possible to say what type of mosaic the mottling on S. 42667 here represents. Additional inoculations with this mosaic type are in progress to determine its identity.[8]

As disclosed in series 4 and 5, which represent 32 tuber grafts of S. 42667 on mild-mosaic 41956, no evidence of infection resulted. Likewise, in

[8] Inoculations with this type of mosaic made by the writers after the manuscript of this paper was submitted indicate that the mosaic type may be the same as latent mosaic.

series 8, involving tuber grafts of healthy Green Mountains on S. 42667, including a random sample of 100 tubers from tops grown adjoining mild-mosaic Green Mountains, no mild mosaic resulted, while in series 7 the control graft of healthy Green Mountain on mild-mosaic 41956, 9 in 20 tubers, or 45 per cent of the grafted tubers, developed mild mosaic. Although S. 42667 developed mosaic symptoms (type not determined) besides top necrosis in a few of the tuber grafts, these results indicate that this seedling manifests resistance to mild mosaic in the tuber grafts also.

In 1931, a naturally fertilized progeny of S. 42667 was grown in the disease test plots between rows of mild-mosaic Green Mountain plants. Although this progeny was produced from seed that resulted from natural fertilization, it is safe to assume that a very high percentage of the seed was the result of self fertilization or its equivalent, since the fruits were obtained in a comparatively large plot of this seedling with no other good pollen-producing varieties in close proximity. Four hundred and sixty-four selections of this progeny were grown in this test. At harvest 3 tubers from each hill were reserved for planting and observation the following season. Table 3 gives the data obtained.

It will be seen that only 7 per cent of these seedlings showed mild mosaic. It will be remembered that S. 42667 resulted from a cross between 2 seedlings, S. 24642 and S. 40568, both of which were apparently resistant to mild mosaic under ordinary field conditions. In the same field-contact test of 1931, a naturally fertilized progeny of seedling 43752, which had only

TABLE 3.—*Potato seedlings and Green Mountain, exposed to mild-mosaic Green Mountain in field test plots. Presque Isle, Maine, 1932*

Parentage of seedlings	Variety	Lots			Hills in mosaic lots		
		Total	Mosaic		Total	Mosaic	
			No.	*Per cent*		*No.*	*Per cent*
S. 42667 naturally fertilized	464 seedlings	464	33	7	842	138	16
S. 43752 naturally fertilized	75 seedlings	75	21	28	550	101	18
S. 42672 × S. 42667	101 seedlings	101	27	27	754	119	16
S. 43322 × S. 42667	77 seedlings	77	34	44	971	192	20
	Green Mountain controls	54	54	100	1584	1061	67

one resistant parent, *viz.*, 40568, was grown. Twenty-eight per cent of the selections from this progeny showed mild mosaic. A small progeny of a cross between 2 resistant sibs, S. 42672 and S. 42667, was also included in these tests. Twenty-seven per cent of the seedlings in this progeny showed varying degrees of infection with mild mosaic in 1932. (Table 3). Another progeny in these tests was that of a cross between a parent that developed a few mild-mosaic plants in the plant-breeding nursery, S. 43322, and the most resistant parent yet available, S. 42667. It will be seen from the data in table 3 that this progeny does not show the same degree of resistance as the other progenies. In this case 44 per cent of the seedlings were infected with mild mosaic. These progenies will be given another test before final conclusions are drawn. The results, however, are quite significant when a comparison is made between the percentage of seedlings of each of the progenies that contracted mild mosaic and the control plots of Green Mountain. The data in table 3 show that every plot of these Green Mountain controls developed mild mosaic. It is evident, then, that a high percentage of the seedlings in each progeny tested is more resistant than Green Mountain in field tests.

DISCUSSION

The data for the tests of leaf roll and spindle tuber are not conclusive, as seasonal variation in the spread of these 2 diseases in the Green Mountain checks grown under field conditions is so variable. The results show, however, that most of the seedlings are susceptible to both of these diseases. A few of them show a low percentage of infection for the years in which they were grown, but it will be seen that the Green Mountain checks for the corresponding years also showed a similar condition, so that the low degree of infection can not be considered an indication of resistance.

Varieties differ in their reaction to streak. Spaulding No. 4 manifests the symptoms of this disease in a milder form than Green Mountain. According to Atanasoff (1), some varieties are masked carriers of streak. Since differences in reaction such as those noted exist, it ought to be possible to produce varieties that will be very resistant to this disease.

The reaction of different potato varieties to streak may be grouped as follows: (*a*) Latent or masked carriers. (*b*) Varieties manifesting mild necrosis on the foliage. (*c*) Varieties manifesting severe dwarfing, curling, and necrosis on tops that usually die before tubers are developed.

Field tests with 2 other viroses, *i.e.*, latent mosaic and mild mosaic, have shown that wide differences exist between varieties in their reaction to each of these diseases. The Green Mountain variety is quite susceptible to both of these diseases under conditions prevalent in northern Maine. One seedling, S. 41956, has been produced that is quite resistant to latent mosaic. Field-contact tests, leaf rubbing, and tuber grafting failed to produce a plant carrying this virus. It is, however, susceptible to mild mosaic but

fewer of the plants contract the disease than is true of the variety Green Mountain. Another seedling, S. 42667, is extremely resistant to mild mosaic and rarely contracts latent mosaic in the field tests, but it becomes infected with latent mosaic in the more severe tests when the disease is expressed in the plant as top-necrosis. Others are found that are as easily infected as the Green Mountain.

The reaction of the different potato varieties and seedlings to latent mosaic may, therefore, be grouped under three heads, (*a*) varieties highly resistant to latent mosaic as S. 41956, (*b*) varieties resistant in field tests but contracting latent mosaic in tuber and shoot grafts and manifesting the disease as top necrosis as S. 42667, (*c*) symptomless or masked carriers. Although varieties like S. 42667 readily develop top necrosis in tuber- or shoot-graft inoculations, they rarely contract this disease in contact with symptomless carriers under field conditions. It has been found that latent-mosaic carriers like Green Mountain seedlings contract latent mosaic more easily, apparently through contact with diseased foliage, than do seedlings manifesting this disease as top necrosis. It is probable that in the presence of the insect vector, yet to be found, latent mosaic will be a serious factor in varieties manifesting it as top necrosis. Otherwise, such seedlings may be propagated with the same degree of success as the highly resistant group and remain essentially free from latent mosaic, even if grown in contact with masked carriers of the disease. This statement is supported by the fact that the seedling cited as an example of the group that develops latent mosaic as top necrosis has been grown for 10 years in the plant-breeding plots. During this time not a single case of top necrosis has developed in spite of the fact that in one year, at least, it was grown in a row adjoining a Green Mountain row.

The reaction of different potato varieties and seedlings to mild mosaic may be grouped under the following heads: (1) Varieties or seedlings highly resistant to mild mosaic in all tests. (2) Seedlings that seldom contract the disease in the field but become infected as the result of tuber grafts. (3) Seedlings that become infected but manifest milder symptoms than those found on Green Mountain. (4) Seedlings that contract the disease as readily as Green Mountain and express symptoms similar to those found in mild-mosaic Green Mountains. These differences between seedlings are plainly evident but the number of factors responsible for them is not so clearly defined. Green Mountain has been used as the source of mild-mosaic inoculum, and, since most Green Mountain plants harbor a complex of 2 or more viruses, the results are somewhat complicated. Some of the seedlings tested for mild mosaic are possibly resistant to latent mosaic carried by the Green Mountain and, therefore, show milder symptoms. In field tests many seedlings may escape infection, and, yet, mild mosaic may be transmitted to them by means of tuber grafts. It is probable that such

seedlings harbor morphological or physiological characters that in some manner interfere with infection or inoculation by insect vectors. Whatever the factors involved may be, it is apparent that such seedlings can be maintained free from mild mosaic with considerably less effort than Green Mountain in northern Maine.

It is quite evident that some of the factors involved are genetic in nature and that resistance and susceptibility to mild mosaic are heritable. This conclusion is substantiated by the results obtained from a cross between 2 seedlings resistant to mild mosaic in the field. In the F_1 progeny seedlings were obtained that were more resistant to this disease than either parent, and others that were more susceptible. One of the hightly resistant seedlings of this progeny, No. 42667, was inbred and an F_2 progeny was obtained. Four hundred sixty-four of these seedlings were tested for their reaction to mild mosaic in 1931 and 1932. The 1932 data show that about 7 per cent of these seedlings became diseased, while, under the same conditions, healthy Green Mountains planted as checks contracted the disease in 100 per cent of the plots. Two or more genetic factors, cumulative in effect, are necessary to interpret these results. It should be pointed out here, however, that the number of plants of some of these seedlings was less than those grown in the Green Mountain control plots in 1930. For this reason a number of the seedlings may have escaped infection. Further tests will be made to determine this point.

In the early years of breeding for resistance to mild mosaic the main object was to produce varieties of economic importance that possessed resistance to this disease; therefore, only the selections that were apparently resistant to the virus in the plant-breeding plots were given further tests in the disease test plots. Here they were grown in contact with mild-mosaic Green Mountains or given the more severe tests, such as tuber grafting, etc. As a result, the data from 1924 to 1930 are rather fragmentary. Beginning with 1931, however, fewer progenies have been tested in the plant-disease plots but larger numbers of each progeny have been grown. In these cases the seedlings were not previously selected for resistance or susceptibility to the virus disease.

It might be mentioned here that the first objective of this program, *viz.*, the production of varieties of potatoes of economic value resistant to mild mosaic, has been accomplished. One of these, S. 42667, which has been named Katahdin (3) has been given extensive tests in a number of States and to a considerable extent it has been distributed to commercial growers through the cooperating experiment stations.

Our experience has shown that, although certain potato seedlings may readily be infected with mild or latent mosaic by means of tuber grafts, nevertheless, these same seedlings apparently are resistant to infection under field conditions. In connection with this experience it may not be

amiss to recall the common experience with leaf roll of potato. It is well known that attempts to transmit leaf roll to healthy potatoes by the leaf-rubbing method of inoculation have failed. Certain aphids, however, readily transmit this disease. It then would seem reasonable to suppose that certain varieties could easily be infected by artificial methods, but would resist infection from insect vectors.

Artificial methods of infection give, among other things, valuable information regarding immunity, symptoms, conditions favoring infection, etc., but such methods are not necessarily infallible criteria regarding the reaction of plants to disease under natural field conditions.

It also has been indicated that potatoes may be resistant to infection from insect vectors but not necessarily to artificial methods of infection, such as tuber or shoot grafting.

It is realized that these investigations on resistance of potato to mosaic were not conducted with pure cultures of viruses. The Green Mountain variety, the source of infection for the field-exposure tests and for many of the tuber grafts, harbored latent mosaic in combination with mild mosaic. The "healthy" Green Mountain controls carried latent mosaic in masked form. However, in some of the inoculations seedlings carrying only mild mosaic, which, in combination with latent mosaic, produced the typical mild-mosaic symptoms on Green Mountain were used. Preliminary observations indicate that mild mosaic and latent mosaic may be caused by more than two viruses.

The recent work of Burnett and Jones (2), Johnson (5), Koch (6), Murphy and McKay (8), Quanjer (10), Smith (15), Salaman and Bawden (11), and Valleau and Johnson (16) has confirmed the former contention and findings by Murphy (7), Quanjer (9), and Schultz and Folsom (13) that there are several types of mosaic of potato and has extended these findings, showing by analysis and synthesis that more than one virus is involved in potato mosaic. Although it may be difficult to develop a potato that is resistant to all of the reported viruses, our results show genetic segregation for resistance to certain of the mosaics and we may conclude that it is possible to produce varieties resistant to many if not all of these diseases.

From the evidence of several viruses and possible virus complexes that may infect the potato and from the possible menace of masked carriers of viruses to nonresistant varieties, the potato grower might have adequate grounds for concern about the fate of this important crop. Fortunately, however, potato localities vary regarding the presence and spread of virus diseases; it is uncommon to find all the reported viruses in a single potato section, usually only a few of them requiring serious attention. In northern Maine, mild mosaic in Green Mountain; in southern Maine and sections south of Maine, leaf roll; and in some of the western States spindle tuber and rugose mosaic apparently are the viroses that require primary consid-

eration in control programs. Furthermore, vectors of viroses vary from one locality to another and from season to season in the same locality.

Latent mosaic probably is harbored more generally in important commercial potato varieties in our country than any other virosis. However, as has been pointed out, most of these varieties are masked carriers of this disease so that, unless latent mosaic occurs in combination with other mosaic viruses, it apparently produces only minor effects in these carriers.

With the indication that varieties resistant to latent and mild mosaic can be developed, satisfactory control of at least 2 important virus maladies of potato seems assured.

SUMMARY

Because of the variable conditions of infection, the test for resistance to leaf roll and spindle tuber are inconclusive. None of the varieties or seedlings included in the tests were immune from these diseases.

Differences in reaction of Spaulding No. 4 and Green Mountain to streak were found, the symptoms being expressed in milder form in the first-named variety.

Three types of reaction of different potato varieties to streak were observed: (1) Latent or masked carriers, (2) varieties manifesting mild necrosis on the foliage, (3) varieties showing severe dwarfing, curling, and necrosis on tops that usually die before the tubers are formed.

Seedling 41956 was found to be highly resistant to latent mosaic. In field tests S. 42667 also was resistant to this virus but contracted the disease in tuber-graft tests, where is was expressed as top necrosis. Symptomless or masked carriers of this disease include the Green Mountain and other well-known varieties.

The reaction of different potato varieties and seedlings to latent mosaic may be grouped as follows: (1) Highly resistant varieties, (2) varieties resistant in field tests but contracting latent mosaic as top necrosis in tuber grafts, (3) latent or masked carriers.

The reaction of different potato varieties and seedlings to mild mosaic may be grouped as follows: (1) Highly resistant, (2) seedlings that seldom contract mild mosaic in the field but become infected in tuber grafts, (3) seedlings manifesting milder symptoms than Green Mountain, and (4) seedlings contracting mild mosaic as readily as Green Mountain and expressing the symptoms of this disease as clearly as this variety. Seedlings that had previously been selected in breeding-plot tests for possible resistance to mild mosaic when subjected to more severe tests in the disease test plots showed lower percentages of mosaic than the Green Mountain checks grown under the same conditions.

The results of a cross between 2 resistant parents showed transgressive inheritance, some of the seedlings being more resistant than either of the parents, others more susceptible.

More work will have to be done before a complete genetic analysis can be made, but the results so far indicate that there are 2 or more genetic factors involved that are cumulative in effect.

The principal objective of the earlier work, which was to produce varieties of economic importance resistant to mild mosaic, has been accomplished. One of the seedings, S. 42667, which has been named Katahdin, has shown a high degree of resistance to mild mosaic in all of the tests. This resistance is combined with other characters that are very desirable from the economic standpoint.

Observations on the reaction of potato to mild and latent mosaic have shown that potato varieties that may readily be infected by artificial methods seldom contract these diseases in the field, which indicates that artificial methods of inoculation are not necessarily infallible criteria regarding the reactions of plants under field conditions.

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A PHYSIOLOGIC FORM OF SEPTORIA TRITICI ON OATS[1]

RODERICK SPRAGUE[2]

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INTRODUCTION

A Septoria leaf-spot disease was found on fall-sown oats (*Avena* sp.) in the Alsea River Valley in western Oregon during the winter months from December, 1930, to March, 1932. It was present both in commercial fields and experimental plots. It was moderately destructive on oats growing in a highly acid (pH 4.8) red soil derived from sandstone and shale. No Septoria leaf spot occurred on varieties of wheat, barley, rye, or spelt growing adjacent to heavily infested oats. A species of Septoria was found on velvet grass (*Holcus lanatus*) in February, 1932. The host and parasite were both prevalent. The fungus on the host and in pure culture is nearly the same as the Septoria on oats. Cross inoculation studies are in progress. The disease also occurred, but with less severity, in the Willamette Valley, adjacent to the Alsea Valley.

Acid soil, open winter weather, and heavy rainfall are predisposing factors in the Septoria leaf spot. The disease may be serious, and, as observed, it seems to be moderately important in small areas on some of the acid soils of western Oregon.

The slender pycnospores and slow, yeastlike growth on potato-dextrose agar differentiate the fungus from *Septoria avenae* Frank. The latter is the only species of Septoria previously noted on oats in the United States.

HISTORY

The literature on *Septoria avenae* was reviewed by Weber (10), who showed that this fungus has rather short, cylindrical pycnospores. He found the ascigerous stage of that fungus, which he named *Leptosphaeria*

[1] Cooperative investigations conducted by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Oregon Agricultural Experiment Station.

[2] Assistant Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The writer is indebted to Dr. G. F. Weber, of the Florida Agricultural Experiment Station, for diagnosing material, and to Dr. S. M. Zeller, of the Oregon Agricultural Experiment Station. He thanks Prof. H. P. Barss, of the Oregon Agricultural Experiment Station, and Dr. A. G. Johnson, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, for constructive criticism of the manuscript. Dr. Johnson obtained literature, photographs of original *exsiccati* and the original Desmazières materials and writings sent by Dr. Foëx from France at the time Dr. Weber was conducting studies under Dr. Johnson's direction at the University of Wisconsin.

avenaria, and that it grew moderately fast in culture in contrast to the characteristically slow, yeastlike growth of *S. tritici* Desm. (10, 11).

Septoria tritici was described by Desmazières (2) in 1842, as pointed out by Weber (11), on wheat collected by M. Roberge. In 1843, Desmazières (3) described *S. graminum* on dry leaves of grasses (*in foliis siccis graminum*), stating that it was similar to *S. tritici;* in 1847 he (4) reduced *S. tritici* to varietal rank under *S. graminum,* making it *S. graminum* var. *B. tritici,* and he added variety *C. avenae* on oats. He gave the spore length of variety *B. tritici,* but he merely stated that variety *C. avenae* was identical with the type *S. graminum.* Weber (11) concluded that the Septoria on wheat leaves in the United States was identical with Desmazières' original *S. tritici* but that it was distinct from *S. graminum,* the latter having obscure pycnidia and very slender pycnospores less than 1.5 μ wide. Weber (11) gave *S. tritici* specific rank and suggested that *S. graminum* probably was confined to wild-grass hosts. Weber (10) also stated, after examining type material of *S. graminum* var. *C. avenae,* that this fungus was distinct from *S. avenae,* especially in the more slender spores of the former. Weber (10, 11) did not secure infection on oats with *S. tritici* from wheat nor on wheat with *S. avenae.*

In Europe two species of Septoria have been reported on oats, namely, *S. avenae* and *S. graminum.* According to Gram and Thomsen (7), *S. avenae* produces dark spots on the leaves. Septoria has been reported on oats from many widely separated localities, most writers referring the fungus to *S. graminum.* Cunningham (1) mentions *S. graminum* on wheat and oats from New Zealand; Marchionatto (8) lists *S. graminum* on oats from the Argentine; Miège (9) refers to *S. graminum* on both wheat and oats from Morocco; and Garbowski (6) lists both *S. graminum* and *S. glumarum* Pass. on dying leaves of oats from Poland.

Diedicke (5) described *Septoria planiuscula* on *Avena planiuscula* (Mycotheca March Nr. 1750). The pycnospores were from 3.5 to 4.5 μ wide by 30 to 45 μ long, with seven septa, making *S. planiuscula* clearly distinct from the species herein described.

SYMPTOMS

The lesions most frequently develop near the leaf tips but occur on any leaf part, including the sheath. The lesions are circular to elongate. (Fig. 1, A and B.) In many cases the fungus progresses longitudinally between restricting leaf veins and forms long, somewhat rectangular lesions. The lesions are gray with indefinite or yellow margins of various intergradations. (Fig. 1, B.) Most of the yellow or greenish yellow host tissue occurs at the distal end of the lesion, and, in cases where the spot covers most of the width of the leaf, the greenish yellow area also includes all of the leaf beyond the lesion. Obviously, this is attributable to the obstruc-

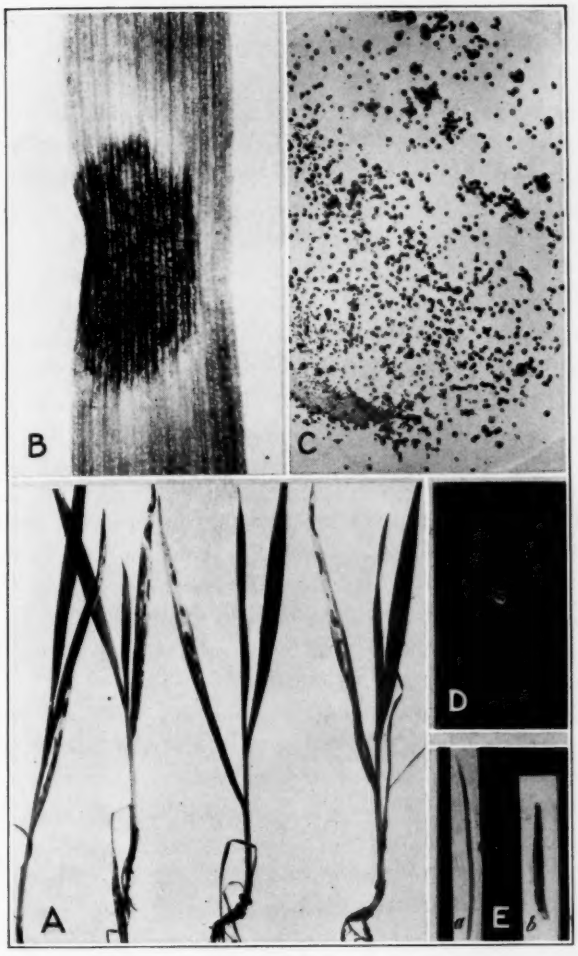

FIG. 1. A. Plants of Red Rustproof oats from Alsea River Valley, Oregon, February, 1931, heavily infected with *Septoria tritici*. B. Enlarged view of a lesion on Red Rustproof oats from Alsea River Valley, Oregon, February, 1931, showing light-colored border, shrunken host tissue, and pycnidia of *S. tritici*. ×4. C. Portion of a potato-dextrose agar Petri-dish culture of *S. tritici* isolated from wheat from Willamette Valley, Oregon, and grown 3 weeks at 15° C. The plate was inoculated with a spore suspension. ×¾. D. Portion of a potato-dextrose agar Petri-dish culture of *S. tritici* isolated from Red Rustproof oats from Alsea River Valley, Oregon, and grown 10 days at 15° C. The plate was inoculated with a transfer of mycelium. E. *a*, pycnospore of *S. tritici* from Red Rustproof oats from Alsea River Valley, Oregon, February, 1931. ×423. *b*, Pycnospore of *S. avenae* Frank from oats, Puslinch, Ontario, Canada, August 9, 1915. (Fungi Columbiani, No. 4771.) ×423.

ting action of the lesion. There may be as many as 10 or more lesions on a leaf blade, but usually there are from 3 to 5. (Fig. 1, A.)

The pycnidia are very prominent on the lesions and occur in parallel rows between the veins of the leaves, with their bases attached or adjacent to the veins. (Fig. 1, B.)

During the winter of 1930–31, Septoria leaf-spot lesions were first evident in December, increased in number until early February, and rapidly diminished in number in March as the Septoria-infected leaves rotted away and were replaced by new, noninfected growth. The winter of 1930–31 was mild, moderately damp, and followed in early spring by several weeks of exceptionally dry weather. During midwinter, due to the abundant lesions, the growing oats plant had a scorched appearance. Counts made at this time showed that approximately 40 per cent of the leaves had one or more Septoria lesions. *Helminthosporium avenae* Eidam also occurred on the leaves.

CAUSAL ORGANISM

Pure-Culture Studies

The following pure cultures were used in comparative studies on various culture media:

1. Septoria isolated from Red Rustproof oats, *Avena byzantina,* in the Alsea Valley, Lincoln County, Oregon, Feb., 1931.
2. Septoria isolated from Winter Turf oats, *Avena sativa,* in the Willamette Valley near Jefferson, Oregon, March, 1931.
3. *Septoria tritici* Desm. isolated from winter wheat:
   a. from fresh host material supplied in the spring of 1931 by E. Gram, Lyngby Experiment Station, Denmark;
   b. from a collection made in the spring of 1931 at Corvallis, Oregon; and
   c. from a collection made in the spring of 1931 at High Prairie in eastern Washington.

Comparative studies of these cultures were made chiefly on potato-dextrose agar (pH 5.5) in test tubes and in Petri dishes.

Vegetative cells and spores from young cultures were used for inoculating the agar slants in test tubes. Most of the Petri-dish cultures were made by pouring water dilutions of the pycnospores over the surface of poured plates (Fig. 1, C).

Cultures 1 and 2 were identical. They also were similar to all available cultures of *Septoria tritici* isolated from winter wheat. Colonies from water dilutions of pycnospores on the surface of agar plates formed glistening, flesh-pink spore masses after incubation for several days at 20° C. These colonies consisted mainly of masses of spores in various stages of formation and germination. The hyphae in these colonies were at first fragmentary, and as the colonies grew older, pigmented hyphae with thicker

walls developed and finally covered the entire colonies with a black convoluted stroma or scurf. For a time the flesh-pink spore masses broke through the stroma, but eventually the colonies became masses of carbonaceous mycelia.

The Septoria isolated from oats (cultures 1 and 2) grew very slowly (Fig. 1, D) and transfers to test tubes required several months to cover the surface of agar slants incubated at 15° C. In similar cultures grown in Petri dishes the dark macrohyphae and the pigmented hyphae grew beyond the main body of the colony in aster-like rays for a distance of 1 mm. or more. These stellate-margined colonies also were very evident in Petri dishes inoculated by water dilutions of pycnospores.

When the cultures were several weeks or months old they had a tendency to produce white, cottony, aerial mycelia over the earlier-formed stromatic masses. This was common on cultures 1 and 2 from oats but less pronounced on cultures 3a, 3b, and 3c from wheat.

All transitions from pseudopionnotes to well-formed pycnidia were found in pure cultures, the latter being found after transfers on potato-dextrose agar had grown for several weeks at 15° C. In some cultures, pycnidia developed in the agar below the surface, and, being accompanied by only a small amount of vegetative hyphae, were easily seem.

On sterile Melilotus stems in test tubes the spores were formed in naked masses, acervuli, and malformed pycnidia.

No ascigerous stage was discovered in cultures isolated from oats grown in the Alsea River Valley. Sterile corn meal, Melilotus stems, potato-dextrose agar, and wheat grain were inoculated with pycnospores and with mycelium of the Septoria isolated from oats. Cultures were incubated in the ice box and out of doors for several months, but no ascigerous stage was found. A Leptosphaeria was occasionally found at the base of old overwintering oats stubble in the field but attempts to isolate it were unsuccessful. The writer questions the connection of the Leptosphaeria with the Septoria from oats.

In studying the characteristics of spores grown in pure culture it was necessary to deal with fresh transfers grown at fairly uniform temperatures of approximately 15° C. Old colonies or those subjected to widely different conditions produced spores with widely different characteristics, such as vacuolation and distortion of various sorts. Some colonies after being incubated in an ice box for four months bore masses of spores that superficially resembled Fusarium conidia.

Artificial Inoculations

During the winter of 1930–31, cereals were grown in the greenhouse in pots of soil from the Alsea River Valley and inoculated, by means of atom-

izers, with spore suspensions of culture 1. Winter Turf oats (*Avena sativa*) inoculated and incubated under a bell jar at 50° F. developed typical lesions and pycnidia. Goldcoin wheat similarly treated did not become infected.

In October, 1931, extensive inoculations with culture 1 were made on plants of various cereals and cheat grass 4 to 12 weeks old and incubated in a modified Wisconsin-type spray chamber. The plants were grown in 6-inch pots, 8 plants to a pot; and there were 2 to 4 pots of each variety. The plants were left in the chamber for 16 days. In the first series of inoculations negative results were obtained on all these plants. It was found, however, that the water supplied to the chamber was being inadvertently warmed to 19° C., and after repiping the chamber to permit use of unheated water at temperature of approximately 10° C., infection was obtained on various varieties of oats. The results are given in table 1. Infection is given as positive (+) in the table when one or more plants showed pycnidia in typical lesions. Reisolations were made from each host that became infected. All uninoculated checks remained free from infection. Infection occurred mainly at the tips of leaves and also in other areas on the lower leaves. Data on infection show that the fungus is not a virulent

TABLE 1.—*Results of inoculating 6-week-old cereals with pycnospore suspensions of Septoria culture 1 and incubating in a spray chamber for 16 days*

Plants inoculated		Results
Genus and species	Crop and variety	
	Oats	
Avena byzantina	Red Algerian	+
Do.	Nortex	+
Do.	Red Rustproof	+
Avena sativa	Green Russian	+
Do.	Iogold	+
Do.	Silvermine	+
Do.	Winter Turf	+
Avena sativa orientalis	White Tartar	+
Avena strigosa	Sand	+
	Barley	
Hordeum vulgare	Tennessee Winter	−
	Rye	
Secale cereale	Common Winter	−
	Wheat	
Triticum vulgare	Goldcoin	−
Do.	Huston	−
Bromus tectorum	Cheat grass	−

parasite and that it apparently requires comparatively cool conditions for optimum development.

Morphology

The morphological characteristics of Septoria on oats from the Alsea River Valley, Oregon, are as follows:

The pycnidia are formed in substomatal cavities, their bases being firmly attached to the side of a vein. The pycnidia are amber brown to black and vary somewhat in shape, most of them being globose to subglobose, or ellipsoid with the long axis parallel to the length of the leaf. They are from 40 to 100 μ wide by 60 to nearly 150 μ long. The ostioles in mature pycnidia tend to be oval and differ greatly in size, being from 5 to 25 μ wide by 10 to 36 μ long, averaging 12 μ wide by 20 μ long.

Pycnospores are slender with the ratio of length to width more than 20 to 1. (Fig. 2.) They are narrowly cylindrical, sometimes slightly

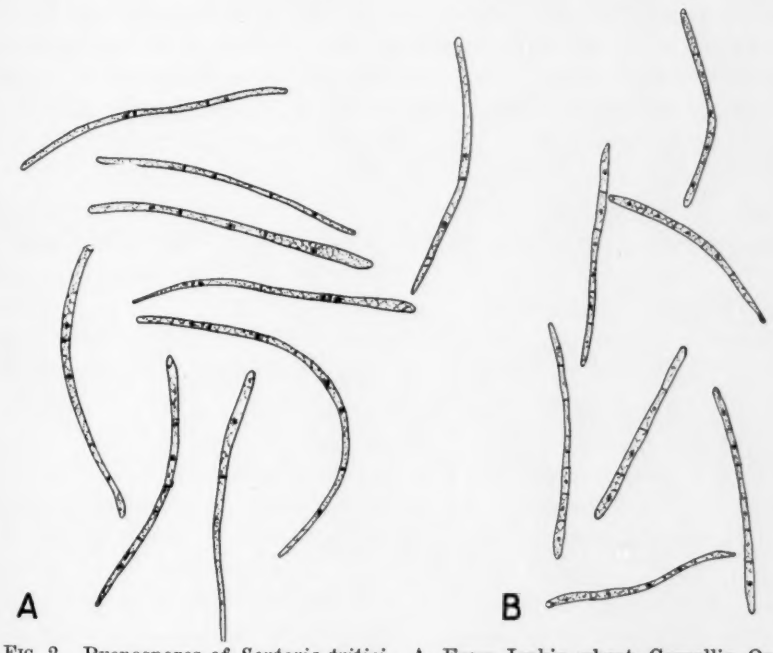

FIG. 2. Pycnospores of *Septoria tritici*. A. From Jenkin wheat, Corvallis, Oregon, March, 1931. ×500. B. From oats from Alsea River Valley, Oregon. ×500.

swollen at the base, straight to greatly curved, hyaline, and 1–7-septate (usually 2–5-septate). The ends are rounded and the contents homogeneous, with nuclei and nucleoli clearly evident. The spores are from 1.6 to 3.5 μ wide by 35 to 120 μ long, averaging 2.3 μ wide by 68.1 μ long, 2.1 wide by

67.6 μ long, and 2.2 μ by 80 μ long, respectively, in three collections made during February and March, 1931.

The mycelial development on agar cultures has been discussed. In the host cells, the mycelium is hyaline to yellow or may even be deeply pigmented. It averages approximately 2 μ in diameter.

Taxonomy

There are several outstanding differences between *Septoria avenae* Frank and the Septoria on oats from western Oregon. The pycnospores of *S. avenae* have a length-breadth ratio of 8–12 to 1 compared with a ratio of usually 20 to 1 or more for the Oregon Septoria. (Fig. 1, E.) The two fungi produce entirely different types of colonies on culture media and, finally, there is no ascigerous stage known for the oat Septoria from the Alsea River Valley, Oregon, while *S. avenae* is genetically connected with *Leptosphaeria avenaria*.

Type material of *Septoria graminum* var. *C. avenae* Desm., No. 1708 Desmazières, plantes Cryptogames de France Fasc. xxxv. 1847, was examined from material originally sent by Dr. Foëx from France to Dr. A. G. Johnson, Bureau of Plant Industry, U. S. Department of Agriculture. In addition, the corresponding specimen of Desmazières (No. 1708) *exsiccati* in the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, was examined. The first-mentioned material had amber brown pycnidia that were elliptical in cross section and measured 30 to 78 μ wide by 48 to 108 μ long (55 by 80 μ). The pycnospores ranged from 0.8 μ wide by 16 μ long to 1.2 μ wide by 32.1 μ long (1.1 by 30 μ). Apparently they were nonseptate, but many of these old and brittle spores fractured into three portions as if cross walls might have been forming at the time they were collected. It was suspected that the material sent by Dr. Foëx and seen by Weber (10) had immature spores. The untouched specimen of Desmazières (No. 1708) in the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, showed pycnidia very similar in size and arrangement to those on oats from Oregon. They measured 41 to 82 μ wide by 70 to 120 μ long. Approximately 60 per cent of the pycnospores in these well-developed pycnidia were one to four or more septate. These spores measured 0.9 to 2 μ wide by 28 to 58 μ long (1.54 by 38.88 μ).

These measurements of *Septoria graminum* var. *C. avenae* Desm. are sufficiently close to those of Septoria on oats from Oregon to justify including both fungi under the same name. Most of Desmazières' No. 1708 is immature, and this, combined with its great age and shrunken condition, has led to some confusion in the past.

Desmazières' type collection of *Septoria graminum* on wild grass (No. 1328 plantes Cryptogames de France) has dark-brown pycnidia that are nearly circular in cross section and measure 45 to 100 μ wide by 45 to 103 μ long, but most of them are uniformly spherical and close to 50 μ in diameter. The spores of Desmazières' *S. graminum* on grass (No. 1328) from material in the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, and from material in Dr. Johnson's possession, are all nonseptate, so far as it is possible to determine from their present condition. The spores are slightly yellower than the hyaline ones of *S. graminum* var. *C. avenae* and are distinctly shorter and narrower and are frequently less curved than many of the spores of this variety on oats. The differences in spores and pycnidia indicate that the two fungi are distinct. A comprehensive study of many Septoria cultures and specimens on grasses will be necessary to determine the exact nature of *S. graminum* Desm. on these grass hosts. It is doubted if it occurs on oats; at least, no specimens have been seen on this host that resemble Desmazières' fungus on wild grass.

The Septoria on oats from Oregon, which is identical with *S. graminum* var. *C. avenae* Desm., logically falls under *S. tritici* on account of almost identical morphological and cultural characters. Dr. Weber, who examined one of the collections from the Alsea River Valley, Oregon, stated, in a letter dated February 28, 1931, that he considered that the pycnospores of the submitted material on oats were within the range of *S. tritici* and quite distinct from *S. avenae* Frank. On account of the sharp division in host range, but only slightly smaller spores (Fig. 2), the form on oats is considered a distinct physiologic form. It must be emphasized that *S. tritici* on wheat has not been known to attack oats nor does the physiological form on oats from Oregon attack wheat.

The writer believes that probably all of the foliicolous Septoriae mentioned in literature on oats are either *S. avenae* Frank or *S. tritici* (form on oats).

A technical description of *S. tritici* on oats follows:

Septoria tritici Desm., physiologic form on Avena (Syn.: *S. graminum* var. *C. avenae* Desm.) not *Leptosphaeria avenaria* Weber (*Septoria avenae* Frank).

Description

Lesions on leaves and sheaths gray, irregular or rectangular, partly restricted by veins, indefinite or bordered by greenish yellow areas, which frequently involve the entire distal portion beyond the lesions; pycnidia prominent, scattered in rows parallel to the veins, subepidermal and substomatal with bases appressed to the veins, subglobose to globose, or ellip-

soid with long dimension paralleling main veins, 40 to 100 μ wide by 60 to 145 μ long, averaging (February collection) 74 by 95 μ, walls of pycnidia amber brown to (sometimes) black, smooth, composed of polygonal cells; ostiole oval or sometimes circular 5 to 25 μ wide by 10 to 36 μ long (12 by 20 μ); pycnospores exuded in short, pale, flesh-colored cirrhi, cylindrical hyaline, with septa 1 to 7 frequently indistinct, 1.6 to 3.5 μ wide by 35 to 120 μ long (2.2 by 70 μ), ratio of pycnospore length to width usually greater than 20 to 1.

The hyphae within the host cells are yellow to olive brown and average 2 μ in diameter.

On *Avena sativa* and *A. byzantina* in western Oregon and on *A.* sp. in France; material was collected near the junction of Alsea River and Five Rivers in Lincoln County, Oregon.

Collections have been deposited in the Oregon State College Herbarium, Corvallis, Oreg., and in the Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

SUMMARY

A locally prevalent Septoria leaf spot was found on cultivated oats growing in an acid soil in the Alsea River Valley in the coastal region of western Oregon. The disease was most destructive during the months of December, 1930, to February, 1932, inclusive.

Because of its slender spores and slow, yeastlike growth in culture, the fungus is distinct from *Septoria avenae* Frank and is morphologically very similar to *S. tritici* Desm. It has been designated as a physiologic form of *S. tritici* Desm. A full technical description is given.

The form on oats is identical with *S. graminum* var. *C. avenae* Desm. and the latter is distinct from *S. graminum* Desm. on grass. The form on oats did not attack wheat and the form or forms on wheat so far have not been found on oats.

It is suggested that all the reported cases of Septoria on leaves of oats probably are either this physiologic form of *S. tritici* on oats or *S. avenae* Frank.

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THE EFFECT OF SULPHUR FUNGICIDES, APPLIED DURING THE BLOOM, ON THE SET OF APPLE FRUITS

L. H. MacDaniels and A. B. Burrell

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INTRODUCTION

These studies supplement a previous publication[1] in which the literature was reviewed. On the basis of the data then presented it seemed evident that sulphur, if in contact with the pollen, inhibited its growth on agar media; that sulphur applied to the stigmas before or shortly after pollination reduced the set of fruit; and that sulphur, applied 24 to 48 hours after pollination, had little effect on the set. Since that time, additional work in the orchards at Ithaca, in the Champlain Valley, and in western New York has been carried on to obtain more data bearing on the problem. The points given particular consideration were, 1st, the effect of lime-sulphur spray applied during full bloom as compared with sulphur dust; 2nd, the length of time after pollination before sulphur dust or lime-sulphur spray could be applied without reducing the set; 3rd, the effect of the application of these fungicides to whole trees under orchard conditions; and 4th, the length of time necessary for the pollen tube to traverse the style of the apple blossom under different temperature conditions, as tested by a method other than sulphur application. It is felt that publication of the data at this time is appropriate because of renewed interest in spraying or dusting during bloom in the control of apple scab and other diseases.

EXPERIMENTS

In the first series of experiments, 6 Northern Spy trees 20 years of age, in a cultivated orchard, were chosen for comparable vigor. On each of these trees 50 vigorous spurs were selected, the blossoms thinned to 2 per spur, and the spurs enclosed in glassine bags. Four different treatments were given to each tree, there being 10 to 12 bags including 10 to 22 spurs in each treatment. All blossoms were pollinated with Rome pollen on the same day. Dusting sulphur was applied to the stigmas at the intervals indicated in table 1. Temperature was favorable for pollen-tube growth throughout, the maximum being 81°, 88°, 82°, and 82° F. on successive days.

It is to be noted in this series that the average per cent set increases directly with the interval between pollination and application of sulphur.

[1] MacDaniels, L. H., and J. R. Furr. The effect of dusting-sulfur upon the germination of the pollen and the set of fruit of the apple. N. Y. (Cornell) Agr. Expt. Sta. Bul. 499. 1930.

TABLE 1.—*Set of fruit as affected by the application of dusting sulphur to stigmas at time of pollination and at intervals after pollination. Northern Spy, 1932. Set after June drop in per cent of spurs treated*

Interval hours	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Tree 6	Ave.
0	13	45	47	26	33	21	31
24	25	56	31	31	25	36	34
72	76	66	46	56	68	53	61
Check	72	70	89	63	80	82	76

The most significant difference is between the 24- and 72-hour interval. In all cases the set on the spurs receiving sulphur immediately or within 24 hours after pollination was much less than the pollinated check and in all but one, less than that on the spurs that received sulphur after the 72 hour interval. Table 2 gives the results obtained on 5 McIntosh trees chosen in the same way as the Northern Spy trees referred to in table 1. Here, with one exception, the application of sulphur either before or coincident with pollination reduced the set as compared with the checks.

TABLE 2.—*Set of fruit as affected by the application of dusting sulphur to the stigmas at different times with relation to pollination. McIntosh, Spring, 1932. Set after June drop in per cent of spurs treated*

Treatment	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Ave.
Sulphur and pollen applied at the same time	17	17	43	21	33	26
Sulphur applied 24 hours after pollen	47	20	44	23	17	30
Pollen applied 24 hours after sulphur	37	5	29	18	22	22
Pollen only applied	39	20	64	30	53	41

In a third experiment, unit branches about an inch in diameter were covered with 3×6 ft. cheesecloth bags. Pollen was applied with a small brush. The sulphur was blown on with a duster. The results are summarized in table 3. Here again, the application of dust before the pollen gave the greatest reduction in set. In another trial with McIntosh in 1930, 2 hand-pollinated bagged unit branches set fruit on 38 to 43 per cent of

TABLE 3.—*Set of fruit on bagged hand-pollinated unit branches as affected by dusting with sulphur. Variety McIntosh. Spring, 1932*

Treatment	Tree	No. blossom spurs	Per cent growing points blossom	No. spurs with fruit Sept.	No. fruits Sept.	Per cent blossom spurs with fruit Sept.
Pol. May 24	1	87	75	27	28	31
Dust May 24	2	81	57	41	44	50
Average						40.5
	1a	87	52	46	63	53
Pol. May 24	1b	120	85	72	88	60
Dust May 25	2	72	50	43	51	60
Average						57.6
Pol. May 25	1	45	33	14	16	31
	2a	84	71	27	32	32
Dust May 24	2b	131	78	18	18	14
Average						25.6
Pol. May 24	1	56	35	32	45	57
No dust	2	113	63	45	50	40
Average						48.5

their blossoming spurs as compared with 20 per cent on a comparable branch dusted immediately following pollination.

The results of the most extensive experiments with unit branches are given in table 4. In this series the unit branches were chosen for their uniformity, on 13 different trees, and covered with 3×6 foot cheesecloth bags, as in the preceding series. The pollen was applied by hand, the dust with a crank-type duster, and the lime-sulphur spray (1–40) with a 3-gallon hand sprayer. On the bagged branches either lime-sulphur spray or dust applied immediately following pollination greatly reduced the set of fruit. Application after 24 hours reduced the set considerably, but differences at longer intervals were not significant. On the uncovered branches the application of lime-sulphur spray reduced the set 20 per cent as compared with the checks.

In another series of experiments, groups of 8-year-old trees, chosen for uniformity and adjacent position, were dusted and sprayed at various times as indicated in table 5. The trees were somewhat small for their age and were growing in alfalfa sod. They were, however, in fair vigor. The small volume of the crop for trees of this age is in large part due to the small size of the apples, caused by late season drought.

Although the comparison between whole trees at this early bearing age is likely to be unsatisfactory because of the natural variation in the amount

TABLE 4.—*Set of fruit on unit branches as affected by the application of lime-sulphur spray and sulphur dust during full bloom. McIntosh, Spring, 1932*

Treatment	No. branches	Bagged (B) or open (O)	Per cent blossom spurs with fruit June	No. fruits per 100 blossom spurs June	Per cent blossom spurs with fruit Sept.
Check	13	O	64	93	25
L. S.	5	O	40	48	20
Pollen only	13	B	71	115	37
P.+L. S. at once	5	B	30	35	13
P.+L. S. 24 hours	1	B	65	91	28
P.+L. S. 27–29 hrs.	9	B	54	79	28
P.+L. S. 48 hours (in one case 53 hrs.)	5	B	68	107	30
Pollen only	13	B	71	115	37
P.+S. dust at once	2	B	28	34	13
P.+dust 24 hrs.	1	B	56	81	26
P.+S. dust 27–29 hrs.	9	B	68	108	38
P.+S. dust 48 hrs.	3	B	60	100	29

of crop borne, certain things stand out. In all 5 groups, lime sulphur applied on May 26 greatly reduced the set as compared with the adjacent checks. Trees dusted both on May 26 and May 27 also showed a much poorer set than the adjacent checks. The application of lime sulphur or dust on May 27, however, showed no significant reduction of set. It should be pointed out in this connection that variation in results on whole trees in an experiment of this sort is very likely to occur because of the difference in the stage of bloom of different trees on the same day. As indicated be-

TABLE 5.—*Set of fruit on whole trees as affected by spraying with lime-sulphur and dusting with sulphur during full bloom. Eight-year-old McIntosh. Spring, 1932. (Yield in bushels per tree)*

Treatment		Group 1	Group 2	Group 3	Group 4	Group 5
May 26	May 27					
Lime sulphur		.3	.4	1.3	1.7	1.7
	Lime sulphur	.8	1.5			
Dust	Dust	.5	.3			
	Dust	1.0	.8	1.5	2.0	
Dust				2.0	2.5	
None	None	.9	.6	1.8	3.0	2.2

fore, the stage of bloom when sulphur is applied is very important, as fungicides applied in the early stages of bloom reduce the set much more than those applied late after pollination has occurred.

Since the effect of sulphur in reducing the set of fruit depends on the time of application with reference to pollination, it is of value to know the length of time required for the pollen-tube to grow down through the styles and accomplish fertilization. To get data on this point, a series of experiments was laid out, adapting the method used by Sandsten.[2] Trees were chosen for their comparable vigor and 50 vigorous spurs selected on each tree. Each spur was thinned to 2 blossoms and covered with a glassine bag. All the blossoms were pollinated on the same day and the styles were cut off at the base with a pair of sharp scissors, at the indicated intervals after pollination. The data presented in table 6 show that McIntosh set no

TABLE 6.—*Set of fruit as affected by cutting off stigmas at different intervals of time after pollination*

McIntosh, 1930

Interval hours	Set on 10 spurs					
	Tree 1	Tree 2	Tree 3	Tree 4		
19	0	0	0	0		
24	0	0	0	0		
30	0	0	0	0		
48	0	2	0	0		
54	3	2	1	0		

Northern Spy, 1930

Interval hours	Set on 10 spurs					
	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Tree 6
25	0	0	0	0	0	0
30	0	0	0	0	0	0
50	0	0	0	0	0	0
66	0	1	0	0	0	0
75	6	1	8	0	0	0
Check	8	6	7	4	5	3

fruits until after an interval of 48 hours, and in 2 out of 3 cases only after 54 hours. With Northern Spy, the first fruit set after 66 hours. Two trees set well after 75 hours, and on 3 trees no fruits set that had the stigmas removed. Difference in the length of time in the case of McIntosh as com-

[2] Sandsten, E. P. Some conditions which influence the germination and fertility of pollen. Wis. Agr. Expt. Sta. Res. Bul. 4: 149–172. 1909.

pared with Northern Spy is correlated with the maximum temperatures, which, in the case of McIntosh, were 74, 74, and 77 degrees on 3 successive days; whereas in the case of the Northern Spy series, the maximum temperatures were respectively 65, 59, 62, and 68 degrees. This series is not altogether satisfactory because of the light set obtained. It is thought this might be explained in part by the fact that the bags were not put back on the blossoms after the stigmas were cut, and hence the bases of the styles dried out sufficiently to damage the flowers. This is borne out somewhat by the series on Northern Spy in 1931, as indicated in table 7. Here the bags were put back over the flowers after cutting the stigmas and a good set was obtained on all spurs with the stigmas cut after an interval of 48 hours. Maximum temperatures in this series were 74, 84 and 83 degrees on successive days.

TABLE 7.—*Set of fruit as affected by cutting off stigmas at different intervals of time after pollination. Northern Spy. Spring, 1931*

Tree	Interval hours	Number bags	Number spurs	Number spurs with fruit	Number fruits	Per cent spurs with fruit
1	22	10	15	0	0	0
	49	15	17	10	17	59
	73	10	12	3	4	25
	Check	10	25	19	30	76
2	22	10	14	0	0	0
	48	15	21	11	12	52
	72	10	12	6	7	50
	Check	10	11	6	8	54

DISCUSSION

The data given above in general confirm the conclusions given in the previous publication.[3] The application of sulphur to the stigmas of flowers on individual spurs did not reduce the set in these experiments so markedly as in the previous experiment probably because of a difference in method. In the previously published work the spurs were thinned to 2 blossoms and sulphur applied to only one of the blossoms on the spur. Under these conditions, practically all of the blossoms treated with sulphur failed to set as they were apparently at a disadvantage in competing with the untreated flower on the same spur.

Considering all data, it seems without question that sulphur applied as dust or lime-sulphur spray, either before pollination takes place or shortly after, reduces the set of fruit. In view of the inhibitory effect of sulphur on pollen germination on agar media, it is perhaps surpising that the set is

[3] See footnote 1.

not reduced more than it is. A possible explanation is that there may be a sufficient number of pollen grains on the stigmas that are not in contact with the sulphur to accomplish fertilization, in spite of the effect of the sulphur upon a large proportion of the pollen grains present. Also, under normal orchard conditions there are usually either enough blossoms that have already been pollinated for some time, and hence not susceptible to sulphur injury, or sufficient unopened blossoms that do not receive sulphur on the stigmas, and hence can be pollinated later, to give a satisfactory crop. There are some indications that application of lime-sulphur spray reduces the set more than an application of sulphur dust. In these experiments spray or dust applied early in the blooming season reduced the set more than the same application toward the end of the blooming season.

From the orchardists' standpoint a moderate reduction in set on varieties normally requiring thinning is sometimes advantageous. It should be pointed out that the varieties used in these tests are self sterile and frequently fail to set because of a lack of pollination. The use of varieties like Wealthy and Baldwin, which usually set when they bloom, might have given different results. It is certain that in many orchards in many seasons trees could be dusted during bloom without serious reduction in the volume of fruit harvested. On the other hand either spraying or dusting in bloom in seasons that are cold or otherwise unfavorable for pollination may reduce the set below a commercial crop.

The data obtained by removing the styles from blossoms at intervals after pollination indicate that under favorable temperature conditions pollen tubes may traverse styles of apple blossoms in 48 hours or less. This coincides in general with the interval after pollination at which sulphur fungicides may be applied without reducing the set.

DEPARTMENTS OF POMOLOGY AND PLANT PATHOLOGY,
CORNELL UNIVERSITY.

DIPLODIA EAR ROT IN INBRED AND HYBRID STRAINS OF SWEET CORN[1]

GLENN M. SMITH AND JOHN F. TROST[2]

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INTRODUCTION

During the field testing of a large number of inbred and hybrid strains of sweet corn in 1930, opportunity occurred to secure data on the relative susceptibility of the different strains to the dry rot of corn, following natural infections by *Diplodia zeae* (Schw.) Lév. Data were taken also from a similar plot of dent corn planted in part of the same field and subjected to the same environmental conditions. It was thus possible to obtain a comparison of the relative amounts of ear infection in these 2 classes of corn.

The dry rot of corn, caused by *Diplodia zeae*, is now recognized as the most serious corn ear rot in the Central States. This fungus causes the most direct and most obvious damage as a mold or rot on the ear, where it seriously affects the quality of the seed. It may attack also the mature plant at various places on the stalk and thereby cause premature ripening of the ear.

This disease occurs in most of the principal corn-growing sections, especially from Nebraska eastward, but has rarely been found in the Pacific Northwest (3). Durrell (2) reported that the losses in Iowa in dent corn in 1921 and 1922 ranged from 3 to 15 per cent of the ears at harvest and caused an average damage to seed corn of 11 per cent. The annual loss in yield of corn in Illinois due to Diplodia seed infection is estimated at 2 per cent of the entire crop by Koehler and Holbert (5).

Losses due to dry rot may occur at 3 stages in the growth of the corn plant, *i.e.*, the seedling, the mature plant, and the ear. Heavily infected seed will ordinarily fail to germinate or, at best, will produce a weak plumule, which is quickly rotted off. Slightly infected seed may germinate but the resulting seedling will be rotted off or the growth will be so retarded that the plants will not develop normally. A high percentage of such stunted plants will produce nubbins, or partially filled ears, or may fail to develop ear shoots. Stalk infections, which frequently result in premature ripening of the plant, are produced from spores coming from pycnidia that

[1] Cooperative investigations, Purdue University Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

[2] Assistant Pathologist and Associate Pathologist, respectively, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

have overwintered on old corn stalks. Burrill and Barrett (1) demonstrated that the spores may be carried considerable distances by the wind. Infections developing on the ear from spores falling into the space between the husks and the ear, under proper temperature and moisture conditions, may spread over the kernels as a mycelial mat and render the ear useless either as seed or food.

Holbert, Burlison, *et al.* (4), have indicated that "differences in resistance and susceptibility to the disease exist in both open pollinated and inbred strains" of corn.

MATERIALS AND METHODS

The results presented in this paper are based on counts of the number of infected ears found in the various inbred lines and hybrids of sweet corn. Very little Diplodia is evident when the sweet corn is in canning condition. Surveys made annually for the last 10 years in Indiana and surrounding States have failed to reveal more than an occasional isolated case of ear infection in roasting ears. On the other hand, the fungus attains its best development when seed corn is being harvested.

In 1930 a number of the better inbred strains and their first-generation hybrids were planted in a field that had been in continuous corn culture over a period of 12 years. During preceding years, the stalks had been left standing on this field until spring, when they were ploughed under as thoroughly as possible with the ordinary plow. However, a rather heavy litter of old stalks, profusely covered with pycnidia of *Diplodia zeae*, was present on the surface of the soil. This inoculum furnished a continuous natural source of spores throughout the season.

In this particular growing season the September and October temperatures were high (September average 65.7° F., 2.1° F. above normal, and October, 53.5° F., 0.81° F. below normal.) Durrell (2) has shown that the optimum temperature for the development of *Diplodia zeae* is between 82° and 86° F., but the fungus develops readily at temperatures above 68° F. Durrell also has shown that "heavy rainfall at the end of the growing period very materially favors the development of *Diplodia.*" The rainfall in September was 2.76 inches and in October 0.89 inch, which was sufficient to support the growth of the fungus.

RESULTS

Comparison of Occurrence of Diplodia zeae on Sweet Corn and Dent Corn

The various strains of sweet corn were all harvested on October 14. The ears from each row, laid at the end of the row, were then classified into 2 groups: (1) clean, bright, and apparently disease-free; and (2) those that

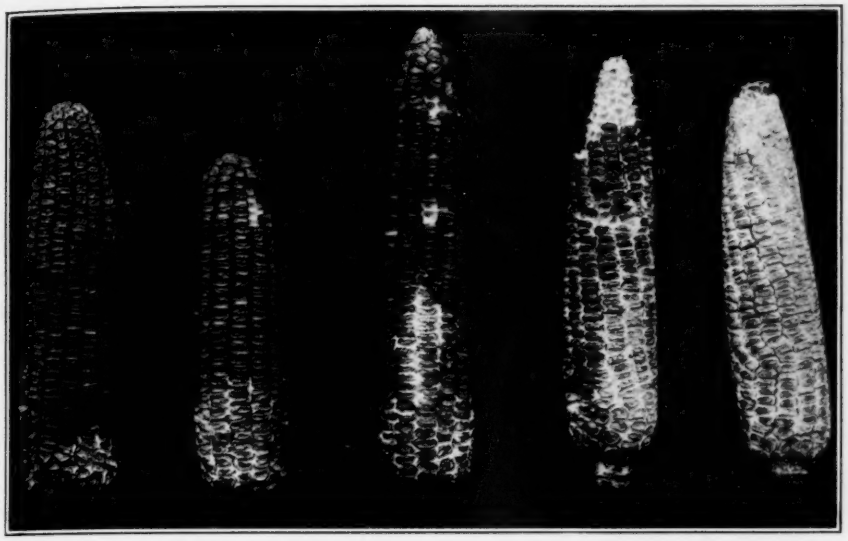

FIG. 1. Ears of Golden Bantam sweet corn showing different degrees of infection with *Diplodia zeae*.

showed any infection with Diplodia. The appearance of sweet corn ears carrying different amounts of infection is shown in figure 1. The ears were then counted and the results are summarized in table 1. A similar procedure was followed for the strains of dent corn, which were harvested 2 weeks later.

Results presented in table 1 indicate a difference of 5 per cent in the amount of natural ear infection in the sweet and dent corns. In the dent-corn group there is an apparent correlation between earliness of maturity and the amount of infection by Diplodia. That is, the early maturing strains of dent corn had relatively high percentages of infected ears and the late types showed low percentages of ear rot. None of the sweet-corn varieties had so high a percentage of infected ears as did the early Yellow Dent strains that matured along with the sweet-corn strains and were subjected to the same exposure to infection.

It has been generally believed that sweet corn is more susceptible to disease than the dent corns. Reddy, Holbert, and Erwin (6) make the following statement: "Sweet corn and dent corn have many diseases in common but in practically all instances the effects are more severe on sweet corn. The varieties of sweet corn of high quality in respect to sugar content are especially susceptible."

Here, where equal opportunity was afforded for these strains to become infected and where so many widely different strains were present, varying from the early maturing strains of Golden Bantam to the latest Narrow-

TABLE 1.—*Comparison of ear infection with Diplodia zeae in inbred and hybrid strains of sweet and dent corn*[a] *at Lafayette, Indiana, 1930*

Kind of corn and variety	Season	Inbreds or hybrids	Number of strains	Average percentage of ears infected
Sweet corn				
Golden Bantam	Early	Inbreds	69	13.0
		Hybrids	21	5.0
Country Gentleman	Midseason	Inbreds	70	9.0
		Hybrids	64	23.9
Early Evergreen	Midseason	Inbreds	11	28.0
Stowell Evergreen	Late	Inbreds	13	12.0
		Hybrid	1	0
Narrow-Grain Evergreen	Late	Inbreds	10	22.0
		Total	259	
			Weighted average	14.8
Dent corn				
Yellow Dent	Early	Inbreds	13	33.0
Reid Yellow Dent	Medium	Inbreds	50	14.6
		Hybrids	37	5.0
Reid and Johnson County White	Late	Inbreds	72	1.8
		Total	135	
			Weighted average	9.5

[a] Data on dent corn kindly supplied by R. R. St. John, Associate Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

Grain Evergreens in sweet corn and from the early dent strains, such as Golden Glow, to the late strains, such as Johnson County White, there were apparently no significant differences in the percentages of ear infection by *Diplodia zeae* in the 2 classes of corn.

Occurrence of Diplodia zeae in Different Strains of Sweet Corn

A more detailed study was made of the occurrence of *Diplodia zeae* among the different strains of sweet corn. A summary of the range of infection among 225 different strains is presented in figure 2. In this figure the strains were grouped into classes differing by 10 per cent; and the heights of the columns indicate the number of strains in each class.

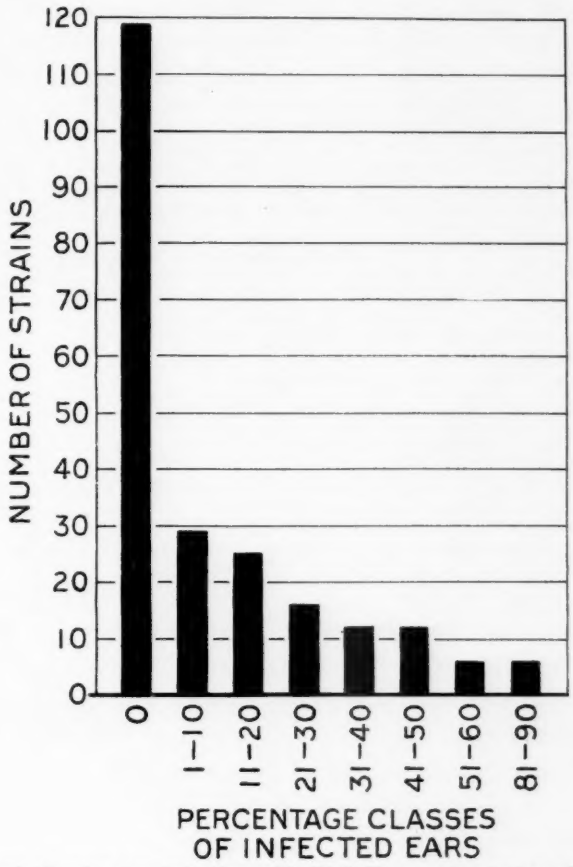

FIG. 2. Graph showing ear infection with *Diplodia zeae* in strains of sweet corn.

According to Koehler and Holbert (5) differences in resistance and susceptibility to Diplodia exist in both open-pollinated and inbred strains of dent corn.

The results presented in figure 2 show that a few of the strains, approximately 10 per cent, developed more than 30 per cent infected ears. Apparently, strains of sweet corn resistant to *Diplodia zeae* may be secured by careful selection among selfed lines under conditions of heavy natural exposure to infection by this fungus. The differences among strains developing side by side and maturing at the same time are significant enough to demonstrate the value of such a method of control.

Comparison of Ear Infection by Diplodia zeae in Inbred Strains of Sweet Corn and in Their First-Generation Crosses

Inbred strains of sweet corn and their first-generation crosses were planted in adjacent rows in a number of these trials. Consequently, it was

possible to make direct comparisons of the percentages of infection in the various selfed lines and in their hybrid recombinations. The results of these comparisons by varieties are summarized in table 2.

TABLE 2.—*Ear infection with Diploida zeae in inbred strains of sweet corn and in their first-generation crosses*

Variety	Number of strains	Average percentage of ears infected in		F_1 hybrids
		Pistillate parent	Staminate parent	
Golden Bantam	20	13.0	15.3	5.0
Country Gentleman	64	12.3	11.9	23.9
Stowell Evergreen	1	35.0	40.0	0

Less infection occurred in crosses of Golden Bantam and Stowell Evergreen varieties, respectively, than in the parent inbred strains of these crosses. However, in the Country Gentleman strains, there was a decided increase in the percentages of infection in the hybrids, amounting, essentially, to a summation of the percentages of the 2 parents.

Influence of Method of Selection on Occurrence of Diplodia zeae in Sweet Corn

Certain inbred lines used in the plot had been selected solely on the basis of canning value during the first 5 years of their inbreeding, while others had also been selected on the basis of disease resistance. A comparison of the percentage of Diplodia-infected ears in these 2 groups in each of 4 varieties is presented in table 3.

TABLE 3.—*Percentages of ear infection by Diplodia zeae in inbred strains of sweet corn. Group I, selected on the basis of canning value only, and Group II, selected for disease resistance and quality*

Variety	Number of strains in		Average percentage of infected ears in	
	Group I	Group II	Group I	Group II
Golden Bantam	16	53	8.3	7.3
Country Gentleman	52	18	18.5	3.3
Narrow-Grain Evergreen	15	28	6.5	3.0
Stowell Evergreen	5	8	12.4	8.7

Under conditions where *Diplodia zeae* annually causes losses in sweet corn, it is apparent from the results presented in table 3 that losses from

Diplodia ear rots can be partly controlled by vigorous selection for resistance. The data show the greatest response to such selection in strains of Country Gentleman, being reduced from 18.5 per cent to 3.5 per cent and the least response in those of Golden Bantam, in which they were reduced from 8.3 per cent to 7.5 per cent. The latter variety usually is considered especially susceptible to disease but, under the conditions of this experiment, showed considerable resistance to infection by *D. zeae*.

SUMMARY

Trials of a large number of strains of dent and sweet corn under conditions of heavy natural infection with *Diplodia zeae* indicate that there is little difference in the average percentages of naturally infected ears in dent corn and in sweet corn.

In these comparisons there was little evidence of any correlation in the percentage of Diplodia-infected ears in the inbred strains and in the first-generation crosses between them.

The results indicate that breeding and selection for resistance will tend to reduce losses from *Diplodia zeae*, particularly in certain varieties of sweet corn.

PURDUE UNIVERSITY EXPERIMENT STATION,
LA FAYETTE, INDIANA.

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PRODUCTION OF CABBAGE SEED FREE FROM PHOMA LINGAM AND BACTERIUM CAMPESTRE[1]

J. C. WALKER

(Accepted for publication March 27, 1933)

Epidemics of cabbage black leg (*Phoma lingam* (Tode) Desm.) and black rot (*Bacterium campestre* (Pam.) E. F. S.) are commonly initiated from inoculum carried with the seed. The writer[2] has previously shown the importance of rainfall in the dissemination of the black-leg organism in the seed bed from foci arising from infected seeds. It was pointed out that the Skagit Valley, Washington, seed-growing region in the vicinity of Puget Sound appeared to be free from this disease. Moreover, experimental plantings of infected seed in that area resulted in little or no development of black leg in seed beds and in seed fields planted therefrom.[2,3,4] This was attributed to the low rainfall of that section during a period of some 3 months after cabbage seed is sown.

Since 1921 many seed lots grown in the Skagit Valley area have been studied under Wisconsin conditions. Although parallel plantings from infected seed showed abundant development of the two diseases, neither black leg nor black rot has been definitely shown to be carried with seed grown in the Skagit Valley. This result prevailed throughout, although certain of the lots tested were known to have been grown from black-leg-infected Wisconsin-grown seed stock.

This paper is a report on certain studies of a similar nature on black rot, and some observations on black leg that confirm previous findings.

RELATION OF RAINFALL TO DISSEMINATION OF BLACK ROT

Black-rot-free seed was inoculated by immersing it in a suspension of black-rot bacteria. This was sown out-of-doors at Madison, Wisconsin, on May 17, 1929, in 2 adjacent rows 12 inches apart. On either side of these, 3 rows of clean seed were sown at the same interval. One-half of each row was covered during rainy periods and the plants were irrigated by running water between the rows with care to avoid splashing. On July 1, when the

[1] Cooperative investigations between the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Dept. Agr. and the Department of Plant Pathology, University of Wisconsin.

[2] Walker, J. C. Seed treatment and rainfall in relation to the control of cabbage black-leg. U. S. Dept. Agr. Bul. 1029. 1922.

[3] ————. Occurrence and control of black leg of cabbage. (Abst.) Phytopath. **10**: 64. 1920.

[4] ———— and W. B. Tisdale. Further notes on the occurrence of cabbage black leg. (Abst.) Phytopath. **12**: 43. 1922.

plants had reached the transplanting stage, they were removed and examined for black-rot symptoms. The data recorded in table 1 show clearly

TABLE 1.—*Relation of rainfall to dissemination of Bacterium campestre in a seed bed. Madison, Wisconsin, 1929*

Row number	Seed inoculated or clean	Exposed to normal rainfall		Protected from rain	
		Total plants	Diseased	Total plants	Diseased
		Number	*Per cent*	*Number*	*Per cent*
1	clean	177	1.7	105	0.0
2	do	250	2.0	175	0.6
3	do	183	14.8	134	1.5
4	inoc.	203	81.8	188	52.1
5	do	[a]		177	63.3
6	clean	101	54.5	157	0
7	do	121	37.2	162	0
8	do	107	7.5	67	0

[a] Plants were not removed at the time final notes were taken, since they were needed later for another experiment; the disease was approximately equal in extent to that in row 4.

that the organism spread readily from the inoculated rows to those adjoining in the unprotected portion of the plot. When the factor of spattering rain was eliminated there was very little spread from inoculated rows. It is evident that spattering rain, as in the case of black leg, is an important means of dissemination of the black-rot organism in the seed bed.

In the Skagit Valley area seed is sown, depending upon the variety, from May 15 to June 15. In general, the rainfall in this area is very light from the middle of May to about September 1. The fact that the dissemination of the black rot organism as well as that of black leg is greatly reduced in the absence of rain suggested an explanation of the general lack of the former in the western Washington cabbage-seed fields. Comparative plantings were made at Mount Vernon, Washington, and at Madison, Wisconsin, in 1931 and 1932.

COMPARATIVE DEVELOPMENT OF BLACK ROT IN WISCONSIN AND WESTERN WASHINGTON

Inoculated seed was sown at Madison on May 13, 1931, and a few days later at Mount Vernon. On July 6, 28 per cent of the plants at Madison showed black-rot symptoms. On July 13, the seed bed at Mount Vernon was examined. No external signs of black rot were found. One plant in about 200 showed slight vascular discoloration when the stems were cross-sectioned.

In 1932 a lot of seed naturally infected with *Phoma lingam* was used. This seed was inoculated with the black-rot organism. Part of it was planted at Madison on May 13 and the remainder at Mount Vernon a few days later. On July 27, 54 per cent of the plants at Madison showed signs of black rot and 18 per cent showed black leg. On July 1, about 1500 plants from the Mount Vernon bed were transplanted. On July 18 the plants remaining in the seed bed and those in the field were examined. In the seed bed only one plant out of several hundred showed unmistakable signs of black rot. In the field no signs of the disease were to be found. On August 31 the field planting was again examined. About 4 per cent of the plants showed systemic lesions of black rot. At no time was there any evidence of black leg found either in the seed bed or in the transplanted field.

From these observations of 2 years it is evident that development of black rot in the seed bed is negligible in the Skagit Valley area. It should be emphasized that the seed in both of these experimental plantings carried a much heavier black-rot inoculum than is ordinarily the case with naturally infected seed.

DISCUSSION AND SUMMARY

Black leg and black rot of cabbage have caused repeated losses due to the introduction of their pathogens with the seed. Ordinarily, the course of development is a progressive one from scattered local centers in the seed bed. This spread is dependent largely upon spattering or wind-blown rain drops. A decade or more of observations of the cabbage seed crop in the Skagit Valley seed-growing area and of the crops grown from such seed crops has substantiated the belief that cabbage seed grown in this area is generally free from the pathogens of the above-named diseases.

This conclusion has been further strengthened by experimental plantings of infested seed in this area. It is believed that the very low precipitation during a 3-month period following the sowing of seed is the most important factor in checking the spread of the parasites.

In recent years a large proportion of commercial cabbage seed production in the United States has been transferred to this area. This change has been stimulated in part by the increasing evidence of its freedom from important seed-borne parasites.

BUREAU OF PLANT INDUSTRY
AND
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

PHYTOPATHOLOGICAL NOTES

Giant Galls Caused by the Root-knot Nematode.—The largest nematode galls ever recorded were observed recently on some ornamental Thunbergias from Florida. Figure 1, B, gives a view of such a gall located at the base of a *Thunbergia laurifolia* Lindl. vine, growing on the grounds of a hotel in Palm Beach in 1925. Similar large galls occurred at the bases of a number of other Thunbergia vines on these grounds. Figure 1, A, represents two pieces from a similar gall on *Thunbergia grandiflora* Roxb., collected in 1933 on a property near Babson Park, where several of these vines have developed these galls within the last few years. Similar galls on this host have been seen repeatedly in peninsular Florida, and Miss Nellie A. Brown kindly supplied a picture of one collected in 1921 in Cienfuegas, Cuba. The gall-like masses of tissue, caused by the root-knot nematode *Heterodera marioni* (Cornu 1879) Goodey, 1932, often reach a diameter of 18 inches to 2 feet, and may attain this degree of development within a period of a few months to a year. Neither of the Thunbergias seems to suffer much from these galls, except perhaps by a reduction in the number of flowers. Older galls soon decay and crumble. In a fresh condition the gall tissue is extremely tough, consisting mainly of lignified fibers. The gall surface is very rugose and of blackish color, whereas the interior has a waxy, yellowish-white appearance. A histological study has not yet been successful because of the extreme hardness of the gall tissues.

Concerning the location of these galls on the host plant, it is remarkable that they do not occur on the root but on the basal stem. They can best be compared with galls produced by the same nema on tubers, corms, rhizomes, and other subterranean stems, with one striking difference, however, that in this case they are formed above ground, as is evident from Fig. 1, B.

Root-knot galls on the basal portions of stems above ground have also been observed on two other plants, and in both instances the whole appearance and character of these galls was similar to that of those on the Thunbergias. The surface is blackish and very rugose, the size also large. Figure 1, E, represents one of this type on the rhubarb, *Rheum rhaponticum*,[1] and Fig. 1, C, on a begonia, *Begonia* sp.[2] From these cases it may be concluded that such giant, extremely rugose, crown-gall-like root-knot galls are probably always basal stem galls. Why it is this region of a plant that responds to an infestation by this nema with such noticeable cancerous

[1] Through the courtesy of Miss Nellie A. Brown, of U. S. Bureau of Plant Industry.

[2] From B. G. Sitton of Shreveport, La., through Dr. F. Weiss, both of U. S. Bureau of Plant Industry.

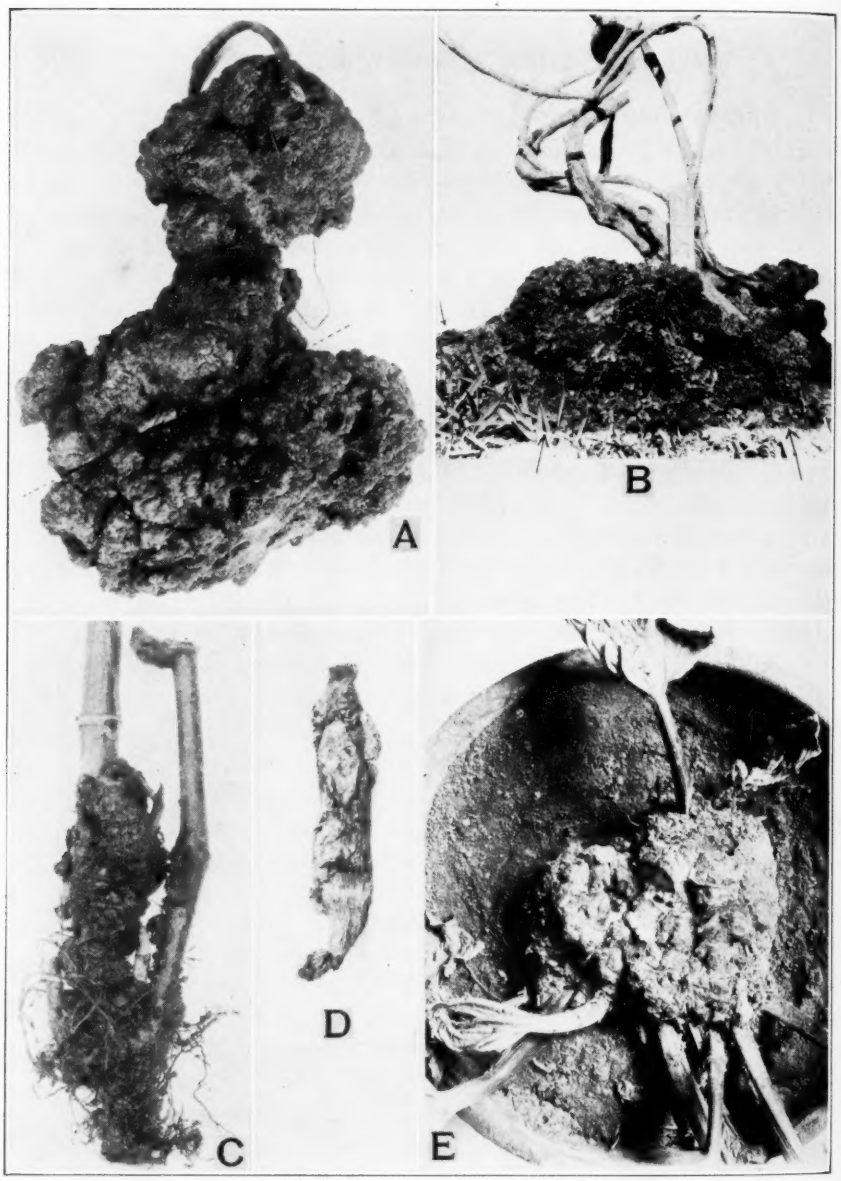

FIG. 1. Giant galls on stems of plants, caused by the root-knot nematode (*Heterodera marioni*). A. Two pieces of such a gall on *Thunbergia grandiflora*. B. The extent of the gall on this *Thunbergia laurifolia* is indicated by the arrows. C. and D. On *Begonia* sp., the latter showing an early stage in the gall development. E. Such a gall on a rhubarb stem (*Rheum rhaponticum*) seen from above.

growth and extreme tissue proliferations, is not yet clear. It may be elucidated by detailed histological and comparative morphological studies of the attacked plants. Figure 1, D, shows a beginning stage in the development of such a gall on the basal stem portion of a begonia. Even this early stage is very unlike the normal root-knot nematode gall on a root; it more closely resembles a crown-gall structure.

The growth of these galls is due mainly to autoinfection, the progeny of the first and succeeding generations of the parasite remaining within the plant. The final result is an enormous accumulation of specimens of the parasite, which, after the final breakdown of the host plant, will invade the surrounding soil.

Another point of interest is the fact that, at least in the case of Thunbergias, the galls usually are fully exposed to the sun. Since the lethal temperature point of larval root-knot nematodes is around 116–117° F., it would appear that autoregulation by the host plant must keep down the temperature of at least the fully insulated portions of the galls, so that this lethal point is not reached. The rugose structure of the galls through increase of the evaporating surface may be favorable to such a function.—G. STEINER, EDNA M. BUHRER, U. S. Department of Agriculture, Washington, and ARTHUR S. RHOADS, Florida Agricultural Experiment Station.

Paraphelenchus maupasi Attacks Hyacinth Bulbs.—Figure 1 represents a cross section through a hyacinth bulb that exhibited disease symptoms produced by a heavy infestation of *Paraphelenchus maupasi* Micoletzky, 1922. Only a few records of the occurrence of this nematode exist. It was first described by Maupas[1] from Algiers but incorrectly referred to *Aphelenchus agricola*. This classification was corrected by Micoletzky,[2] who first placed it in the subgenus Paraphelenchus (1922), which he later[3] (1925) raised to generic rank. De Man[4] described the male but wrongly considered it as that of *Aphelenchus agricola*. In 1929 the writer observed some specimens on coffee roots from Nicaragua. The hyacinth bulbs here described (imported from Holland) apparently represent the first recorded case where this nema was observed in large numbers and was causing definite disease symptoms. As may be seen from the figure, these symptoms

[1] Maupas, E. Modes et formes de reproduction des Nématodes. Arch. Zool. Exper. Sér. 3, **8**: 463–624. 1900.

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FIG. 1. Cross section of hyacinth bulb infested with *Paraphelenchus maupasi.*

are slightly reminiscent of the ring symptoms as produced by other nemas in bulbs. However, in the present case proper rings are not formed because the nema enters the scales of the bulb and does not usually remain in the space between. The scale tissues are much more broken up than in the case of an infestation by *Anguillulina dipsaci.* The disease appears on a cross-cut scale first as a circular yellowish waxy speck. It seems to progress more by way of segments than by following the rings formed by the scales.

In regard to the nematodes it is of interest that no males were seen, although several hundred females were counted.—G. STEINER and EDNA M. BUHRER, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

Disease Symptoms Produced by Anguillulina pratensis in Yams.—Yams from various tropical regions have repeatedly been seen attacked by the meadow nematode (*Anguillulina pratensis* de Man, 1880). In our records the following cases are listed: Three different lots from Japan, one lot each from Jamaica, Porto Rico, and West Africa. The disease symptoms produced by this nematode in yams are small elevations over the entire surface (Fig. 1, A). This figure represents a rather uniformly infested specimen. Under each elevation the tissue containing the nematodes is of brownish discoloration. As the disease advances, the discolored tissue

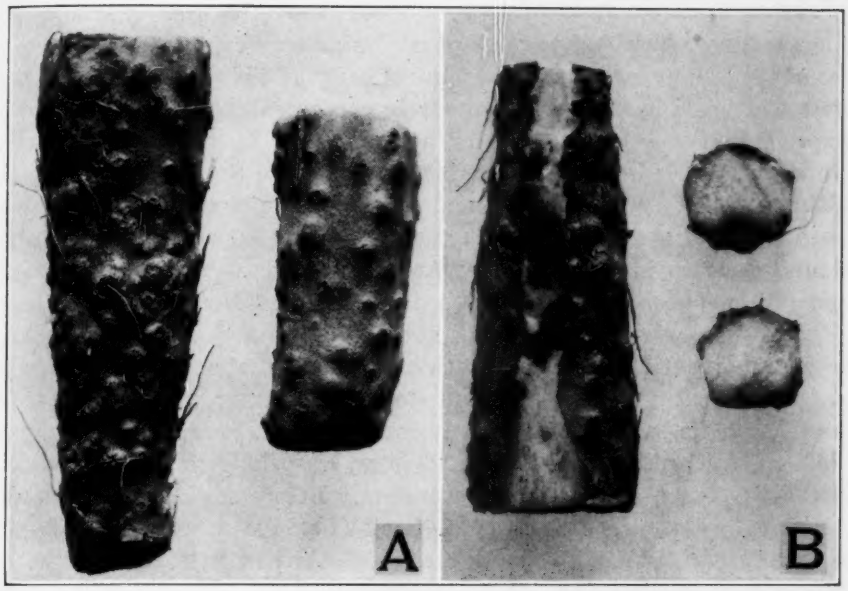

FIG. 1. Yams infested with *Anguillulina pratensis*. A. Surface views. B. Surface view with part of cortex removed to show discoloration beneath surface, and cross sections.

spreads and gradually forms a continuous layer under the surface, penetrating inward simultaneously (Fig. 1, B). General decay then sets in.—G. STEINER and EDNA M. BUHRER, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

Development of Oats Resistant to Smuts and Rusts. The development of strains of oats highly resistant to smut, crown rust, and stem rust, and with desirable grain characters, has been a major objective of the Department specialists interested in oat improvement. Sufficient evidence is now at hand to indicate that this objective has been achieved, at least in so far as combined resistance to all three diseases is concerned. During the crop season of 1933 certain hybrid strains were grown in the cooperative breeding nursery at Ames, Iowa, that continued to show resistance to all three diseases. These lines were obtained from a cross of Victoria × Richland.

Victoria was introduced from South America a few years ago, and is highly resistant to or nearly immune from the various physiologic forms of crown rust. It also is extremely resistant to the smuts of oats but is poorly adapted and, so far, has not been sufficiently productive to recommend it for commercial production. On the other hand, the productiveness and

excellent characters of Richland are well-known. However, it lacks resistance to crown rust and to most physiologic forms of smut. Richland is one of the leading commercial varieties of early oats in the Corn Belt. The cross was made by the senior author in the greenhouse at the Arlington Experiment Farm, Rosslyn, Va., in the spring of 1930. The F_1 plants were grown in the cooperative nursery at the Aberdeen Substation, Aberdeen, Idaho, in the summer of 1930 and were not subjected to any artificial inoculations of smut or rust. In the spring of 1931 seed from these F_1 plants was sown in the nursery at Ames, Iowa, where epidemics of crown rust (*Puccinia coronata avenae* (Corda) Eriks. and Henn.) and stem rust (*P. graminis avenae* (Pers.) Eriks. and Henn.) were developed artificially in separate plantings. At harvest those F_2 plants that showed resistance to one or both rusts were isolated and stored for further study. Seed from these F_2 plants was sown in progeny rows at Ames, Iowa, in the spring of 1932. The F_3 plants were subjected to an artificially produced epidemic of the two rusts, as in the F_2 generation. Before sowing, the seed was inoculated with spores of loose smut of oats (*Ustilago avenae* (Pers.) Jens.) for a test of resistance to this disease also. At harvest several of the F_3 lines showed freedom from smut and appeared to be homozygous for resistance both to crown rust and stem rust.

Seed from several of these more promising F_3 lines was sent to Washington, D. C., for sowing in the greenhouse at Arlington Farm in the fall of 1932. The hulls were removed and the seed was blackened with spores of loose smut of oats collected in the vicinity of Ames, Iowa, in the summer of 1932. Immediately after sowing the seed the temperature of the greenhouse was raised for about a week to facilitate inoculation. In February, 1933, artificially induced epidemics of both crown and stem rust were developed on separate seedings in the greenhouse at Arlington Farm. The inoculation was accomplished by placing the plants in a large glass incubation chamber where they were sprayed with water and dusted with rust spores. They were then allowed to remain in the chamber for 24 hours.

An excellent infection of smut and of the 2 rusts was obtained. Of the several families grown, a few showed a combination of resistance to all 3 diseases. In 1933, selections from these families were tested for resistance to all 3 diseases at Ames, Iowa, where, under field conditions, they continued to show marked resistance to the rusts and smuts.

Heavy epidemics of crown and stem rust were developed in the field each year by the aid of artificial inoculations. Urediospore suspensions of physiologic forms of crown and stem rust, common throughout the North Central States, were hypodermically injected into susceptible plants growing in check rows. These inoculations were initiated when the plants were 8 to 10 inches high and resulted in their complete infection in the check and

border rows when they reached the boot-stage of development. The rust disseminated from these infected rows, combined with the natural infection present, was sufficient each year to produce a maximum infection on all susceptible plants in the nursery.

A second group of selections showing a combination of resistance to all 3 diseases has been developed from the cross Victoria × Richland. Remnant seed from the F_1 plants grown at Aberdeen, Idaho, in 1930 was sown in the greenhouse at the Arlington Farm in the fall of 1931. The F_2 plants were inoculated with urediospores of crown and stem rusts by the method previously described, but, owing to unfavorable conditions, low percentages of infection were obtained. However, progenies from those lines that showed some resistance to the rusts were grown at Aberdeen in 1932 from seed blackened with inoculum containing several physiologic forms of both smuts (*Ustilago avenae* and *U. levis*). At harvest all progenies showing smut infection were discarded. Seed from the remaining plants was hulled and inoculated with smut spores and sown in the greenhouse at Arlington Farm in the fall of 1932. The F_3 plants were subjected to artificial epidemics of both rusts. Excellent infections of both smuts and both rusts were obtained and many plants succumbed; a few families, however, showed high resistance to both rusts and were smut-free. These surviving families were further tested for resistance to all 3 diseases at Ames, Iowa, in 1933 under epidemic conditions in the field. Their descendants continued to show a combination of resistance to crown and stem rust and smut, thus confirming the results obtained from the material continuously tested under field conditions at Ames.

Since the tests of the selections from the cross Victoria × Richland were much more severe in the greenhouse and field than under average field conditions, it is believed that the desired end result, that is, the development of strains possessing a combination of resistance to all 3 diseases has been accomplished. However, it is still too soon to say whether these new oats will be suitable for adoption by the farmer. Their adaptation and productiveness remain to be determined.—T. R. STANTON, H. C. MURPHY, F. A. COFFMAN, and H. B. HUMPHREY, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

The Relative Importance of Cercosporella herpotrichoides and of Leptosphaeria herpotrichoides as Parasites of Winter Cereals.—Until the last few years *Leptosphaeria herpotrichoides* de Not. was generally accepted as the primary parasite involved in the "straw-breaker" foot rot of winter wheat and winter rye in Europe. Recently, in the Pacific Northwest, *C.*

herpotrichoides Fron was shown to be the cause of a straw-breaker type of foot rot of winter wheat.[1]

Foëx and Rosella[2] determined that a fungus, later found to be *Cercosporella herpotrichoides*, was an active parasite of wheat in France.

Recently, Schaffnit,[3] following the work in the United States and in France, found that *C. herpotrichoides* was the cause of the straw-breaker foot rot of cereals in Germany.

In the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, at Washington, D. C., *Cercosporella herpotrichoides* occurs on specimens of diseased wheat collected in Norway by E. Bachala and E. C. Stakman and labeled *Leptosphaeria herpotrichoides*. From conversation with Dr. Olaf Aamodt of the University of Alberta, who has seen the straw-breaker foot rot in Sweden, it is evident that the symptoms of the straw-breaker disease in Sweden are practically identical with those in the United States in Washington, Oregon, and Idaho.

While *Cercosporella herpotrichoides* is an active parasite, the writer has not found *Leptosphaeria herpotrichoides* to be so. Pure cultures of *L. herpotrichoides*, kindly furnished by Foëx from France and by Henry from Canada, have been used in field and greenhouse inoculation studies in the same manner as was *Cercosporella herpotrichoides*.[4] *L. herpotrichoides* grew saprophytically over the basal parts of winter wheat culms and penetrated into the outer leaf sheaths to some extent, but even under the favorable conditions of the experiments it was only a weak parasite.

The writer wishes to point out the need for re-study of the straw-breaker disease in those European countries where no recent work has been done with this malady. It has been the writer's belief for some years that *C. herpotrichoides* is the cause of most of the straw-breaker disease of wheat attributed to *L. herpotrichoides* in Europe.—RODERICK SPRAGUE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Oregon and Washington Agricultural Experiment Stations.

[1] Sprague, R. *Cercosporella herpotrichoides* Fron, the cause of the Columbia Basin foot rot of winter wheat. Science, n. s. 74: 51–53. 1931.

[2] Foëx, Et., and Et. Rosella. Sur les diverses formes du piétin. Rev. Path. Veg. et Ent. Agr. 17: 41–51. 1930.

[3] Schaffnit, E. Beiträge zur Kenntnis der Fusskrankheiten des Getreides (I. Mitteilung). *Cercosporella herpotrichoides* (Fron) als Ursache der Halmbruchkrankheit des Getreides. Phytopath. Ztschr. 5: 493–503. 1933.

[4] Sprague, R., and H. Fellows. The Cercosporella foot rot of winter cereals. U. S. Dept. Agr. Tech. Bull. (In press.)

BOOK REVIEW

Traité de Pathologie Végétale. By G. Arnaud and Madeleine Arnaud. Tome I. Vols. I, II, and Atlas. (*Encyclopédie Mycologique,* Tomes III, IV, V), 1831 pp., 702 figs., 34 col. plates. Paul Lechevalier et Fils, Paris. 1931. 750 fr.

This splendid work on plant pathology of about 1900 pages (including the Atlas) forms Tome I (Volumes I and II, and Atlas) of the authors' treatise, and is also Tomes III, IV and V, of Lechevalier's Encyclopédie Mycologique. It is profusely illustrated with 702 original figures and with 34 colored plates by Mme. S. Ballings. These plates are bound together to form the Atlas. The treatise describes over 400 diseases of the grape vine, fruit trees and small fruits, including subtropical fruits grown in the Mediterranean region. Besides the 400 diseases described more or less fully, many parasitic fungi and bacteria of little or no economic importance, especially in France, are briefly noted. The insects that attack the crops mentioned above and methods for their control are also briefly described. The space allotted to each disease varies from a paragraph to 79 pages (downy mildew of grapes), according to the importance of the crop and of the disease in France. However, all the known parasites attacking the crops mentioned above, in whatever part of the world they occur, are mentioned or described.

The treatise has an introduction of 106 pages. It deals with the history of plant pathology, the classification of diseases according to symptoms, the nature and classification of the parasites (Phanerogamic plants, fungi, bacteria and insects), general control methods, and climatology. It aims to give readers, who are not plant pathologists, sufficient technical knowledge to enable them to understand the cause of disease and the principles underlying control measures, and thus fully profit from the treatment of the diseases in the pages that follow. The introduction is concluded with a long list of monographs on general plant pathology mostly in French, English, German and Italian. Throughout these volumes, each section is followed by an extensive and up-to-date bibliography.

The remainder of Volume I is devoted to the diseases of grapes (543 pages), to a chapter devoted to general considerations on diseases of fruit trees (belonging to the Rosaceae), and to the diseases of apples (335 pages). Volume II deals with the diseases of pear, quince, medlar-tree, mountain ash, hawthorn, peach and almond, apricot, plum, cherry, currant and gooseberry, raspberry and blackberry, cranberry, strawberry, olive, citrus trees, fig, mulberry, pomegranate, loquat, Japanese persimmon, carob, pistachio-tree, jujube-tree, and date-palm. The sub-tropical plants are grouped together under the heading of Mediterranean trees and crops.

The subject-matter is arranged according to the host under its French common name. Common names in English, German and Italian, and the scientific name are also given. In the case of the most important crops, the history of their culture, their economic importance and their botanical classification are dealt with fully. For instance, 95 pages are devoted to these subjects pertaining to the grape vine, and 18 pages to those of the apple. Then follows a list of diseases and insects affecting the host, and a discussion on general control methods. Then, after this, each disease is taken up separately, those of greatest economic importance being dealt with first, and at considerable length. The rest of the diseases attacking that host are grouped as follows: the fungous diseases attacking the leaves, those attacking the fruit, the branches and trunk, and the roots; the bacterial diseases; parasitic insects; non-parasitic (physiological) diseases; and virus diseases.

Each disease is dealt with in a separate section of varying length under the heading of its French common name. Common names in English, German and Italian, and the scientific name and authority are also given. If it is an important disease, its history and geographical distribution, and the susceptibility of varieties to it, are discussed. Then follows the symptomatology, conditions favorable for its development, control measures, and a systematic and morphological study of the organism. The nomenclature receives due consideration, all the synonyms being given, together with their authority. In most cases, a reference to the original description of the organism is included in the bibliography at the end of the section.

These volumes are written in simple, but beautiful French, in order that they may be read by growers and specialists alike. They are therefore easily readable to those with only a slight reading knowledge of this language. They are remarkably free from typographical errors, and are rich in original illustrations and in the extensive and up-to-date bibliographies at the end of each section, throughout the books. The exhaustive treatment of the subjects, the original illustrations, and the extensive bibliographies make this work of great value both as a text-book to the student and as a work of reference to the advanced student and investigator, and should be in the library of every botanical and agricultural institution. The authors are to be congratulated in putting out a work of such size and quality, and on their large number of entirely original illustrations. It is hoped that similar volumes on cereal and vegetable diseases will soon follow these.—H. N. RACICOT, Division of Botany, Central Experimental Farm, Ottawa, Canada.

Announcement

VI INTERNATIONAL BOTANICAL CONGRESS

(Secretary: Dr. M. J. Sirks, Wageningen, Holland)

Wageningen November 6th. 1933

Dear Sir,

The Executive Committee of the VI International Botanical Congress will be much obliged for your kind publishing free of charge the enclosed second press-notice in your journal.

With sincerest thanks, yours faithfully,
the secretary of the Executive Committee
(Dr. M. J. Sirks).

VI INTERNATIONALER BOTANISCHER KONGRESS

(Schriftführer: Dr. M. J. Sirks, Wageningen, Holland)

Wageningen am 6 November 1933.

Sehr geehrter Herr,

Der Vorbereitungsausschuss des VI Internationalen Botanischen Kongress wird Ihnen für eine kostenlose Veröffentlichung der einliegenden zweiten Presse-Mitteilung in Ihrer Zeitschrift sehr verbunden sein.

Mit bestem Dank im Voraus, Hochachtungsvollst,
der Schriftführer des Vorbereitungsausschusses
(Dr. M. J. Sirks).

VI CONGRÈS INTERNATIONAL DE BOTANIQUE

(Secrétaire: Dr. M. J. Sirks, Wageningen, Hollande)

Wageningen, ce 6 novembre 1933.

Monsieur,

Le Comité d'Organisation du VI Congrès International de Botanique vous sera bien obligé de la publication gratuite dans votre périodique de la notice ci-jointe, la seconde notice pour la presse.

Veuillez accepter, Monsieur, nos remerciements anticipés,
le secrétaire du Comité d'Organisation,
(Dr. M. J. Sirks).

VI INTERNATIONAL BOTANICAL CONGRESS

Amsterdam, September 2d.–7th. 1935

The Organizing Committee of the VI International Botanical Congress from various sides has been asked to change the dates of this Congress; the Committee has now decided that the Congress will meet at Amsterdam (Holland)

SEPTEMBER 2D.–7TH. 1935

A first notice regarding this Congress has been sent out to a number of addresses; for additional copies please apply to the secretary, DR. M. J. SIRKS, Wageningen, Holland.

VI INTERNATIONALER BOTANISCHER KONGRESS

Amsterdam, 2–7 September 1935

Der Vorbereitungsausschuss des Sechsten Internationalen Botanischen Kongresses ist von verschiedenen Seiten gebeten worden die Daten des Kongresses zu ändern; demzufolge ist jetzt beschlossen worden, den Kongress in Amsterdam abzuhalten

vom 2 bis zum 7 September 1935.

Eine erste Notiz über den Kongress ist an manche Adressen versandt worden; weitere Exemplare können vom Sekretär, DR. M. J. SIRKS in Wageningen (Holland) bezogen werden.

VI CONGRÈS INTERNATIONAL DE BOTANIQUE

Amsterdam, 2–7 septembre 1935

En conséquence de prières lui venues de divers côtés le Comité d'Organisation du Sixième Congrès International de Botanique a résolue de changer les dates du Congrès à Amsterdam et de les fixer à

2–7 septembre 1935.

Les premières communications concernant le Congrès ont été expédiées. Prière de s'adresser au Secrétaire, M. le Dr. M. J. SIRKS, Wageningen, Hollande, pour en obtenir d'autres exemplaires.

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Official Organ of The American Phytopathological Society

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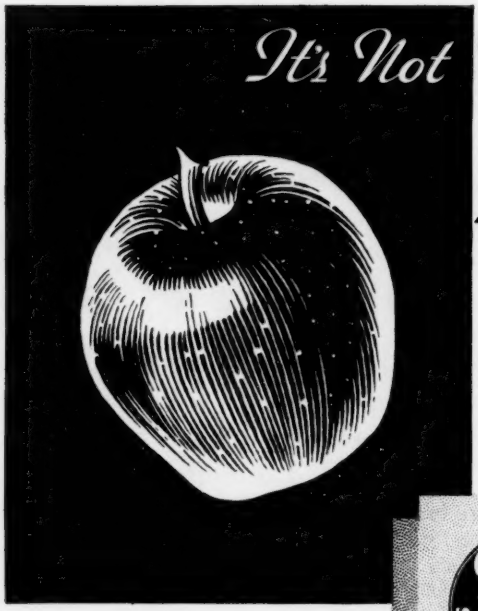

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